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Chemistry, Pharmacology, and Chemical Ecology of Ant Venoms*

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I. THE CHEMICAL AND TOXINOLOGICAL WORLD OF ANTS

Ants as a group contain some of the most specialized as well as the least specialized of social insects. Some species form small secretive, cryptic colonies and prey on only a limited group of organisms (some species of *Cerapachys* and *Leptogenys*) and others live almost anywhere and in a variety

*This chapter also provides information on lethal capacities (Tables III and XV) and pain-producing capacities (Table XVI) of Hymenoptera other than ants.

of nesting materials (*Solenopsis, Monomorium, Wasmannia, Forelius, Camponotus,* etc). Unlike social bees, which, with one known exception, are strictly specialists feeding on pollen and sugar exudates, social wasps, which are predominantly predators, and termites, which are essentially consumers of only cellulose-containing materials, ants are the true omnivores of the insect world. Ants consume living and dead insects of all types, plant materials including seeds, nectar and specialized protein or lipid-rich plant parts, fungi, and insect secretions (honeydew) and even scavenge the flesh of dead and living vertebrates. Ecologically and behaviorally important chemicals, or ecomones, probably affect the lives of social insects more than any other group of organisms, and within the social insects, the ants undoubtedly have used these chemical means the most effectively to increase their own success.

The omniencompassing habits of ants are to a large extent possible because of their successful use of ecomones to communicate biologically meaningful information among colony members, or to members of other colonies and ant species, and to mediate their behavior toward, and defense against, potential predators and competitors. Although ecomones are produced in a variety of formicid glands and serve a multitude of functions, they, with the exception of those found in the venom, will not be discussed here. Further discussions of these interesting topics can be found in various sources (Blum, 1981; Bradshaw and Howser, 1984).

Of all the chemical-producing exocrine glands of ants, the venom glands have exhibited the greatest biosynthetic plasticity and diversity of biological roles. The venoms of social bees and wasps have essentially one function: defense (in the broad sense); the venoms of the solitary Hymenoptera also have one major function: prey capture, with a minor, or absent, function of defense (the bees are not predatory and, hence, their venoms are only used for defense). Ant venoms, however, are used as injectable defensive agents by stinging ants, as topically applied defensive agents by many nonstinging ants, as trail, alarm, sex, queen-recognition, aggregation, attractant-recruitment, and recognition pheromones, as repellents, and even as toxic agents for prey capture. This medley of functions of this most diverse and chemically complicated social organ necessitates that I adopt a somewhat different approach toward ant venoms than has been traditionally followed when discussing animal or plant venoms. Emphasis will be placed on all aspects and functions of ant venoms, including chemistry and activity of venoms used as active defenses as well as the subtle behaviorally mediating aspects of ant venom volatile chemicals.

II. TAXONOMY

The family Formicidae has speciated spectacularly since the Cretaceous period (Wilson *et al.*, 1967) and is divided into at least ten subfamilies, which

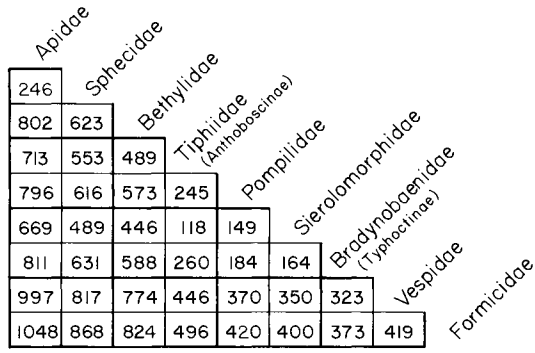


Fig. 1 Measure of taxonomic distinctiveness within the aculeate Hymenoptera showing the phylogenetic separation between the ants and other Hymenoptera [measured in units of taxonomic distinctiveness (DT)]. After Brothers (1975).

include more than 10,000 species distributed worldwide (Taylor, 1978; Gurney, 1975). The systematic relationships between ants and other Hymenoptera remain obscure and there are several theories concerning their evolutionary origin. The two main schools of thought believe that the ants originated either from a line of primitive solitary tiphiid wasps, the Anthoboscinae (Brown, 1954; Wilson *et al.*, 1967), or from the solitary bethylid wasps (Malyshev, 1968). Studies based on both phenetic and cladistic methods of systematics suggest that neither of these groups is closely related to the ants: bethylids are separated from ants by at least 824 units of taxonomic distinctness (DT) and the Anthoboscinae by 496 (Fig. 1). In fact, no existing group is taxonomically closely related to the ants; the nearest group is the Typhoctinae in the Bradynobaenidae (see Fig. 2 for position in the Aculeate phylogeny), which is distinct by 373 units (Brothers, 1975). The minimum taxonomic separation between members of the large branch separating the vespoid wasps plus ants from the bees plus sphecids (see Fig. 2 for a simplified family tree of the Aculeata) is only 339 units. That this value is smaller than the separation between the ants and their closest relative (DT = 373) indicates that the ants are taxonomically very distinct among the aculeates (the closest competitors for that distinction are bees, which are separated from their closest relatives, the sphecids, 246 units). These findings suggest that formicids logically form their own separate group within the Aculeata (Brothers, 1975).

The taxonomic distinctiveness of the ants renders ancestor searching difficult at best. Although the Typhoctinae are the closest living taxon to the ants, this group is specialized. Thus, the true ancestor of the ants was likely to be a form closer to the less specialized Sierolomorphidae (Brothers, 1975 and personal communication).

The evolutionary affinities within the Formicidae are to an extent uncertain, though the family is felt to by monophyletic in origin. A recent family tree

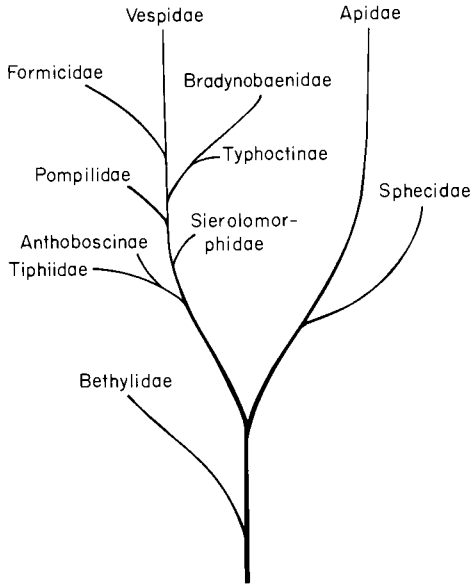


Fig. 2 Simplified family tree of the Aculeate showing major taxa and those considered most closely related to the ants.

of the ants illustrating the relative positions of the subfamilies, together with a survey of the general nature of the venoms within each subfamily, is presented in Fig. 3. Table I lists the taxonomic positions of all the ant genera mentioned in this chapter. A close look at Fig. 3 and Table I illustrates that sufficient information is presently unavailable to enable one to make detailed conclusions concerning the evolutionary origin of the specialized mixtures of venom components elaborated by particular ant species.

III. BIOLOGICAL ROLES OF ANT VENOMS

The evolutionary plasticity of ant venom biochemistry and morphology provided the ants unprecedented opportunities to modify and expand their behaviors and to exploit successfully present and new environments. The plasticity of the sting and venom system was undoubtedly possible because these systems were not essential in ants for any physiological or biochemical processes, or for reproductive roles. That is, because the formicid ancestor no longer required the sting as an ovipositor for injecting eggs into hosts,

Table I

The Classification of Ants (Family Formicidae) including Genera (and Descriptive Common Names) Mentioned in this Review[a]

Subfamily Sphecomyrminae (fossil only)

The formicoid complex
- Subfamily Nothomyrmeciinae
- Subfamily Aneuretinae
- Subfamily Dolichoderinae
  - *Forelius*
- Subfamily Formicinae
  - Tribe Plagiolepidini
    - *Anoplolepis*
    - *Plagiolepis*
  - Tribe Myrmelachistini
    - *Myrmelachachista*
  - Tribe Formicini
    - *Acanthomyops* (citronellal ants)
    - *Formica* (field ants)
    - *Lasius* ("pizmyre" ants)
  - Tribe Camponotini
    - *Camponotus* (carpenter ants)

The poneroid complex
- Subfamily Myrmeciinae
  - *Myrmecia* (bull ants)
- Subfamily Pseudomyrmecinae
  - *Pseudomyrmex*
- Subfamily Ponerinae
  - Tribe Cerapachyini
    - *Cerapachys*
  - Tribe Amblyoponini
    - *Amblyopone*
  - Tribe Ponerini
    - *Dinoponera* (giant ants)
    - *Harpegnathos*
    - *Megaponera*
    - *Pachycondyla*
  - Tribe Ectatommini
    - *Ectatomma*
    - *Paraponera*
    - *Rhytidoponera*
  - Tribe Platythyreini
    - *Platythyrea*
  - Tribe Leptogenyini
    - *Leptogenys*
  - Tribe Odontomachini
    - *Odontomachus*
- Subfamily Leptanillinae
- Subfamily Dorylinae (driver ants)
- Subfamily Ecitoninae
  - *Eciton* (army ants)
- Subfamily Myrmicinae
  - Tribe Myrmicini (broad sense)
    - *Decamorium*
    - *Manica*
    - *Myrmica*
    - *Myrmicaria*
    - *Pogonomyrmex* (harvester ants)
    - *Tetramorium*
    - *Veromessor* (harvester ants)
  - Tribe Solenopsidini
    - *Huberia*
    - *Monomorium*
    - *Solenopsis*
      - *Diplorhoptrum* (thief ants)
      - *Euopthalma*
      - *Solenopsis* (fire ants)
    - *Xenomyrmex*
  - Tribe Pheidolini
    - *Aphenogaster*
    - *Novomessor*
    - *Pheidole*
  - Tribe Ochetomyrmicini
    - *Wasmannia* (little fire ants)
  - Tribe Attini (fungus ants)
    - *Acromyrmex* (leaf cutter ants)
    - *Apterostigma*
    - *Atta* (leaf cutter ants)
    - *Cyphomyrmex*
    - *Sericomyrmex*
    - *Trachymyrmex*
  - Tribe Crematogastrini
    - *Crematogaster*
  - Tribe Leptothoracini
    - *Formicoxenus*
    - *Harpagoxenus*
    - *Leptothorax*
    - *Myrmoxenus*
  - Tribe Dacetini
    - *Serrastruma*

[a]Tribal designations are tentative.

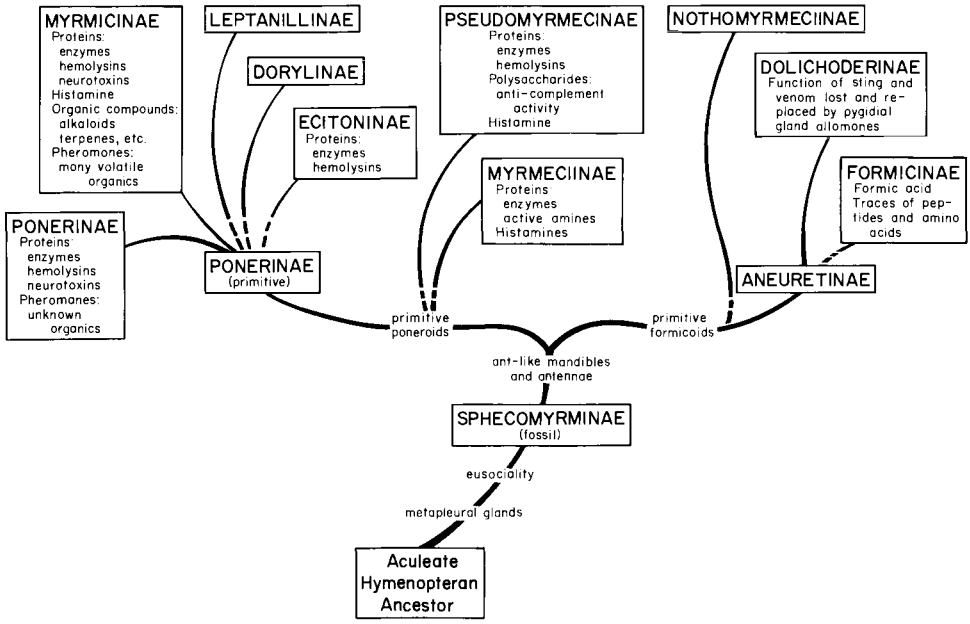


Fig. 3 Phylogeny of the subfamilies of ants (after Taylor, 1978) and a description of their venoms.

the sting and accessory glands were free to take up new roles in response to selection pressures. Thus freed many biochemical and physiological constraints, ant venoms responded to selection pressure by serving in a multitude of new and diverse biological roles.

The results of selection pressures on ant venoms have taken the form of one or more of three functions: offense, defense, and communication. Offensive uses of venoms consist primarily of venom as a means of prey capture (or inactivation) and as a tool for competing with other organisms, including ants, for territory and resources. Defensive uses of venom consist essentially for any of a variety of venom-based means used by ants to defend themselves or their colony against predators of any type. Communication roles of venom consist of any chemical means by which information is communicated from one member of the colony to another.

A. Offense

Information detailing which ant species use their sting in prey capture is often omitted in the literature. Observations and analyses of the actual stinging process itself and its immediate and long-term effects on the prey

are even more frequently lacking. Ants commonly extend their stings when engaged in battle, especially with large prey, and, therefore, observers must be cautious to insure that the sting is actually utilized for prey capture. Also, if prey are experimentally dropped near an ant colony, stinging behavior may reflect defensive use against an intruder and not offensive use for prey capture. Table II is a listing of ant species reported to use their venoms for prey capture or for competing with other organisms. Specific modes of action of some of these venoms are discussed in detail later.

The majority of ant species that use their venoms for prey capture belong to the subfamilies Ponerinae and Myrmeciinae. In many of these, foraging is done by solitary workers. In species that forage on termites or other prey found in groups, recruitment and group raiding seem to be common (Maschwitz and Mühlenberg, 1975; Longhurst *et al.*, 1979b; Hölldobler and Traniello, 1980; Bradshaw and Howse, 1984).

Very little information is available on the use, if any, of venoms for offense by the ants in the subfamilies Pseudomyrmecinae, Dorylinae, Ecitoninae and Leptanillinae. The available information suggests that several myrmicine species that have evolved from the use of venom for prey capture to the use of venom against competitors, especially other ants, have become successful, in part, as a result of the new role of their venom. For example, *Solenopsis geminata* and *Pheidole dentata* are sympatric competitors for some common resources. In these competitors, *Solenopsis* uses its sting (Fig. 4a) as a fighting weapon, and *Pheidole* uses its strong clipperlike mandibles (Fig. 4b) to chop up opponents (Wilson, 1976). For *Solenopsis*, these clashes are partly predatory as well as competitive, for they will, whenever possible, consume the brood of vanquished *Pheidole*. *Solenopsis invicta* uses its venom offensively in a similar fashion against another sympatric species, *Lasius neoniger* (Bhatkar *et al.*, 1972).

The diminutive myrmicine *Wasmannia auropunctata* is often seen as the major ant species in its local environment. When laboratory colonies of *Wasmannia* were challenged with other ant species including *Monomorium* and *Solenopsis*, the introduced ants were often quickly dispatched with a sting (Howard *et al.*, 1982). In natural situations, *Wasmannia* is known to kill or displace colonies of *Myrmelachista ramulorum*, a much larger, aggressive formicine species (Smith, 1936), and held half of a small island in what appeared to be standoff against *Tetramorium simillimum* (E. O. Wilson in Fabres and Brown, 1978). Although none of these studies alone demonstrates that *Wasmannia* uses its sting and venom in natural offensive contexts, their combined observations leave little doubt that this myrmicine has modified its primary offensive use of venom from prey capture to competition with other ant species.

A novel approach in the offensive use of venom is displayed by some species

Table II

Ant Species Known to Use Venom in Offense

Species	Prey/Competitor	Reference
Myrmeciinae		
Myrmecia gulosa	Arthropods	Cavill *et al.* (1964); Robertson (1971)
Myrmecia pilosula	Arthropods	Morrison (1983)
Ponerinae		
Amblyopone pallipes	Insects	Traniello (1982)
Amblyopone pluto	Centipedes	Traniello (1982)
Cerapachys augustae	Termites	Wheeler (1903)
Cerapachys (*turneri* group)	*Pheidole*	Hölldobler (1982)
Harpegnathos saltator	Arthropods	Maschwitz *et al.* (1979)
Leptogenys neutralis	Ant alates, termites, etc.	Wheeler (1933)
Leptogenys attenuata and *nitida*	Isopods	Fletcher (1971)
Megaponera foetens	Termites	Bradshaw and Howse (1984)
Pachycondyla (*Termitopone*) *commutata* and *laevigata*	Termites	Wheeler (1936); Hölldobler and Traniello (1980)
Pachycondyla (*Bothroponera*) *sorror*	Termites	Longhurst *et al* (1980)
Rhytidoponera impressa	Arthropods	Ward (1981)
Myrmicinae		
Decamorium uelense	Termites	Longhurst *et al.* (1979b)
Myrmoxenus gordiagini	*Leptothorax*	Bushinger *et al.* (1983)
Serrastruma serrula	Collembola	Dejean (1980)
Solenopsis invicta	Arthropods, ants	Bhatkar *et al.* (1972)
Wasmannia auropunctata	Arthropods, ants	Fabres and Brown (1978); Howard *et al.* (1982)
Monomorium pharaonis	Insects, ants[a]	Levinson *et al.* (1974); Hölldobler (1973)
Monomorium minimum	Prey[a]	Baroni Urbani and Kannowski (1974); Hölldobler *et al* (1978); Adams and Traniello (1981)
Solenopsis (*Diplorhoptrum*) *fugax*	Ants[a]	Hölldobler (1973); Blum *et al.* (1980)
Nothomyrmeciinae		
Nothomyrmecia macrops	Arthropods	Hölldobler and Taylor (1983)

[a]Venom used as a repellent to drive off workers of other ant species.

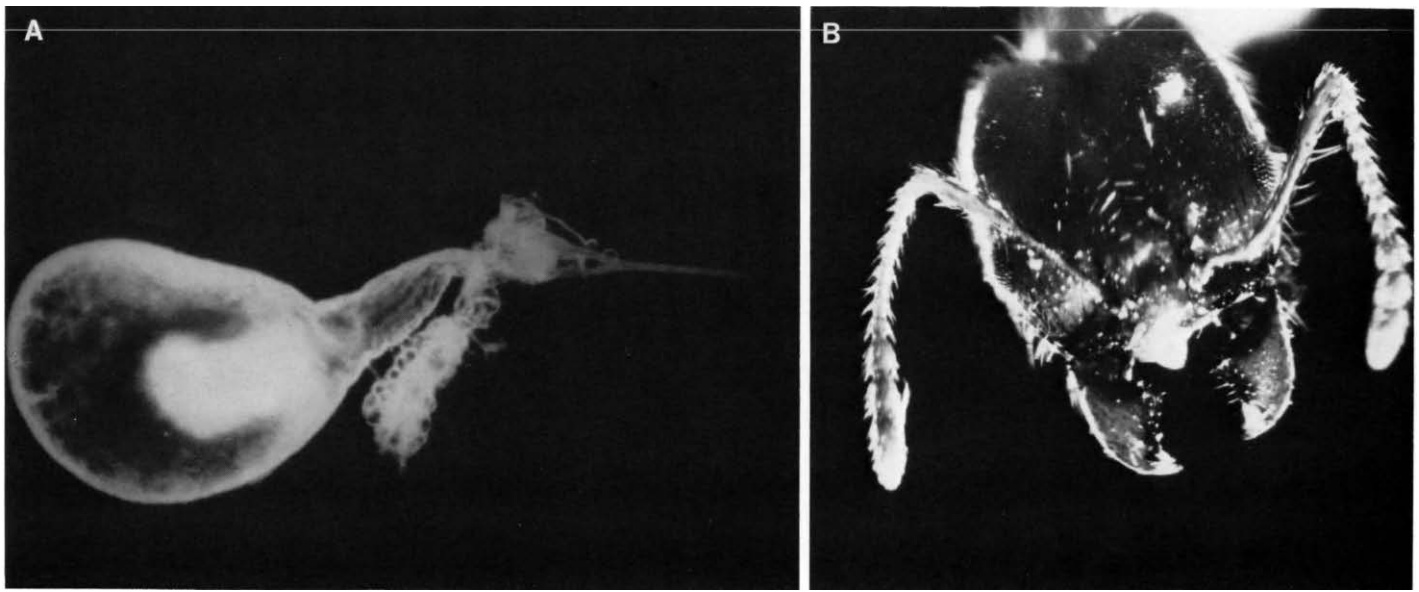


Fig. 4 Fighting weapons of ants (A) The sting aculeus with attached poison (large) and Dufour's (small) gland reservoirs of *Solenopsis invicta*. (B) The cutting/clipping mandibles of the soldier caste of *Pheidole* sp. (photographs by the author).

of *Monomorium* and by the tiny thief ants in the subgenus *Diplorhoptrum* within the genus *Solenopsis*. Workers of some species of both of these myrmicine genera use their venoms as potent repellents to drive off other ant species from either their prey or, remarkably, even from the other ants' own larvae. The prey or host larvae are then carried off and eaten (Hölldobler, 1973; Blum *et al.*, 1980). In the field, *Monomorium* also effectively displaces workers of *Novomessor* and *Solenopsis invicta* with its repellent venom (Hölldobler *et al.*, 1978; Baroni Urbani and Kannowski, 1974). *Monomorium minimum* uses droplets of venom in an offensive stance termed *gasterflag* to interfere with the ability of other ant species (*Lasius neoniger, Myrmica americana, Tetramorium caespitum*, plus others) to feed at a prey item. The repellency of the venom served both to hold possessions of a prey by *M. minimum* and to allow it to displace other species already feeding at a prey carcass. This competitive 'interference' gave *M. minimum* a selective advantage, especially at large food sources and during the hottest part of the day (Adams and Traniello, 1981).

B. Defense

For the vast majority of ant species, defense is the major function of their venoms. Like other social insects, ants are in the position of having a relatively large to huge biomass (the colony) to defend, yet the defenders are small in size. This combination presents unique problems that had to be solved for the evolution of sociality to occur. In particular, a large concentrated biomass of nutrient-rich individuals in a colony presents a potential bonanza for any predator or parasite either large and strong enough or clever enough to exploit the resource. This implies that large vertebrates are now grave potential predators of a colony whereas they are not likely to expend much time or energy attempting to prey on nonsocial species with small individuals.

How can small insects such as ants defend against predators that may be a million times heavier than they themselves? Such predators also often have thick and almost impervious integuments. Against such actual predators, ants use a combination of defensive tactics including mandibles, allomones, offensive taste, behavioral modification (ranging from mass dispersion to alarm, recruitment, and mass attack), and the sting. The sting and venom are ideally suited for this role because, unlike allomones or the small (relative to the predator) mandibles, which often cannot penetrate a predator's integument, the sting is a well-designed hypodermic syringe able to deliver active venom constituents into the living tissue of the predator. Once the venom is delivered into or under the integument, it can more easily affect sensitive and vulnerable tissues. With such an ability, a small animal such

as an ant need not be large to make a defensive impact on even gargantuan predators.

Predators come in a variety of sizes and types as well as employing a mélange of predator strategies. Selection pressure from predators, large and small, on any prey is the sum of all the pressures exerted by all the predators. Thus, prey organisms must respond evolutionarily to minimize the total impact of all predators on their survival and reproductive success. For ants to do this, they must successfully deal with various-sized predators plus competitors. Because defensive tactics are basically different against arthropod and vertebrate predators, each group will be discussed separately.

1. *Venom Use against Arthropod Predators and Competitors*

Arthropod predators of ants are generally similar in size to the ants, or at least are only an order or two in magnitude greater. For this reason, ants are on almost equal ground with these predators, and mechanical and chemical defenses such as mandibles and allomones are effective. Moreover, the sting shaft (the aculeus) does not readily penetrate sclerotized or tough and elastic integuments of arthropods. This is especially true of large ants. Small ants such as *Wasmannia* or *Solenopsis* with their thinner and smaller stings can often, after a period of time, successfully penetrate intersegmental membranes of arthropod predators. For the reason of sting ineffectiveness, the sting and venom of large ants are generally not the primary, or even an important, defense against arthropod predators. The best defenses against arthropods for these ants are the mandibles, the hard integument, their allomonal secretions, and their behavioral abilities to recruit nest mates for mass defense. When venom is used against arthropods, it is generally used as an allomone rather than as an injectable poison. In the entire subfamily Formicinae, this allomonal use is so highly developed that the sting aculeus is lost and venom injection is impossible. The small Myrmicines in the fire ant group of the genus *Solenopsis* have also developed a topically active allomonal venom, though in this case, sting function has been retained. The compositions and activities of these venoms will be discussed later.

A discussion of individual species of arthropod predators of ants will be omitted mainly because, with few exceptions [e.g., some spiders and ants (MacKay, 1982)], generalist arthropod predators do not make a major impact on the reproductive potential of an ant colony. One time in which general arthropod predators do make a major impact occurs during the reproductive flights. At this time, ant alates are often consumed in prodigious quantities by vertebrates and arthropods alike. However, during reproductive flights, the individuals are solitary and have defensive needs similar to those of

ordinary solitary insects. Alate female ants are often ill adapted for defense at this time; they are usually burdened with large stores of nutrients for future establishment of a colony, are often clumsy, and frequently have small mandibles and stings (organs that if large may detract from their future role as a colony reproductive). As venom appears to play very little defensive role in this aspect of ant biology, it will not be discussed further.

One group of arthropod predators are particularly important threats to many ant species. As stated by Auguste Forel (Wheeler, 1910), 'The most dangerous enemies of ants are always other ants....' This statement, whether entirely correct or not, certainly indicates the importance of other ant species as predators or ecological competitors. In most cases of defense against other species of ants, injectable venom is of little use (a few myrmicines, such as *Solenopsis* and *Wasmannia*, constitute exceptions). The ineffectiveness of the primitive sting apparatus is undoubtedly a major reason why many of the successful, populous ant species are nonstinging ants of the subfamilies Dolichoderinae or Formicinae. Many of the most successful myrmicines such as *Crematogaster, Pheidole*, and many of the attines are also nonstinging. In all of these species, plus the army ants (Ecitoninae and Dorylinae), the ineffective sting has been replaced as a fighting tool by powerful well-developed mandibles, strong topically active allomonal venoms (or other glandular secretions), and by agile behaviors. Among the most ecologically successful of the true stinging ants are the fire ants and, they too, have evolved venoms topically active against other ants.

One final use of ant venoms as defenses against arthropods, including other ant species, is the use of venom as a glue. Use of entangling 'glues' as a mechanical means of defense is common among some groups of termites (Prestwich, 1984) but is not generally considered to be a typical means of defense by ants. The best known example of ant-produced defensive sticky substances is that of *Crematogaster*, which uses its Dufour's gland product as an entangling and toxic secretion (Buren, 1958). A little known case of ant use of froth as a defense was reported by Wheeler (1922) for *Pachycondyla* (*Bothroponera*) spp. The origin and detailed use of the froth remained unreported until 1981. Maschwitz *et al.* (1981) then observed that *P. tridentata* and *P. insularis* mixed air, which probably originates from the spiracles on the spiracular plates, with proteinaceous venom to form a froth that effectively entangled attacking workers of *Pheidole*. An individual worker could release ~ 300 mm^3 of venom froth during the course of 20 foam releases. The foam, composed of 99.5% air and 0.5% venom, appeared to act only mechanically, not toxically, when topically applied to attacking ants. When injected via the sting into a large animal (i.e., a human) the venom retained its effectiveness and produced a rather long-lasting pain (Maschwitz

et al., 1981). The venom thus acts in dual roles for defense against both arthropod and vertebrate predators.

2. Venom Use against Vertebrate Predators

Ant venoms can be useful in several different ways as defenses against vertebrate predators. Formicine ants with their reduced stings effectively use their formic acid-rich venoms as an allomonal spray. This spray is particularly effective when directed toward vulnerable areas such as the eyes, nose, or mouth. The corrosive action of formic acid is also effective when applied topically to a wound made by mandibles. This latter method can be rather effective; my own personal experience indicates that the pain and burning of formicine wounds is sometimes as great as that induced by stings from a variety of ponerines or by a superficial honeybee sting.

Gustatory repellency is another venom property of probable defensive value. The acrid taste of formic acid is an obvious example of a venom-based gustatory repellent that is effective against a variety of unspecialized predatory vertebrates. Other venoms also have noisome tastes. These include the venoms of *Dinoponera grandis*, which is bitter, hot, and spicy and leaves a burning sensation in the mouth; *Ectatomma tuberculatum*, which is hot and spicy, numbing, and leaves an intense burning feeling in the mouth; *Pachycondyla obscuricornis*, which is bitter, hot and numbing; *P.* (*Neoponera*) *apicalis*, which is bitter and burning; *Odontomachus hematodus*, which is bitter; and *Pogonomyrmex* spp., which have mildly numbing and burning venoms. Gustatory repellency of ant venoms appears most frequently among ponerine ants, but within this group the venom of the most feared (by humans) species *Paraponera clavata*, as well as that of the army ant, *Eciton burchelli*, have no taste (J. O. Schmidt, unpublished). The value of venom gustatory repellents as a part of the defensive repertories of ants has not been tested and remains unknown.

Venom injection by stinging ants is by far the dominant use of ant venoms as defenses against vertebrates. To be effective against a large predator, a venom once delivered beneath or into the skin must either produce pain or damage. Pain has the obvious advantage that it is a natural part of the animal's physiological warning system, designed to inform that damage might occur, is occurring, or has already occurred. An indication of even potential damage must be taken seriously by an animal. Hence, venom pain serves not only as an immediate punishment, but also as a harbinger of potential damage. Moreover, venom pain provides the potential predator immediate negative feedback relating to its actions. Pain, therefore, is an excellent stimulus to imprint avoidance behavior by the predator.

Venom pain alone may well be a form of chemical Batesian mimicry. Unless followed with tissue damage or other severe consequences, it only tricks the predator into believing that damage will result from the sting. An intelligent predator is capable of learning that pain, like any form of Batesian mimicry, can be false and therefore can be disregarded (beekeepers routinely take this attitude toward honeybee stings).

The other facet of an ant venom *modus operandi* against vertebrates is tissue damage and/or death. Unlike the situation with pain, this venom action is threatening to the predator. Nevertheless, it suffers from the problem that the punishment is delayed rather than immediate. Hence, its action toward the predator is less easily learned. Even if vertebrates do not readily associate sting-induced tissue damage with their predatory assaults on stinging insects, tissue damage can be a subtle, yet probably effective, selection pressure against continued predatory behavior. If the envenomation even slightly reduces the vertebrate predator's survival, or makes the predator less interested in foraging for food, it will constitute a small reduction in the predator's reproductive potential. A vertebrate's own survival can be reduced by venom damage that makes the predator at least temporarily ill or less aware of and able to defend against its own predators. Such a result has been postulated to occur for horned lizards, *Phrynosoma cornutum*, preying on harvester ants (Rissing, 1981).

More overt selection pressure on a predator by venoms includes actual death resulting from massive envenomation or death from allergic anaphylaxis to the venom. Insect venoms are among the most allergenically active of materials. An intriguing unanswered question arises—is this allergenic potential by 'accident' or did selection pressure on the venom act to increase venom allergenicity?

One advantage of sociality is that it allows the evolution of warning and recruitment systems that can be used to launch a mass attack by many individuals against a large predator. Mass attack opens new possibilities for venom activity. If only the venom of a solitary individual defending itself is available for defense, then there is little probability that the venom could become toxic enough to be a real threat to the predator. With the possibility of hundreds of stings being delivered to a predator, a toxic or damaging role of a venom becomes a reality. If this prediction is correct, one would expect to find solitary species of Hymenoptera and those social Hymenoptera with small colony populations to possess venoms of low toxicity, while social species with high populations should possess highly toxic venoms. Table III is a listing of the lethal potentials of some ant and other hymenopterous species against a mammal. Although the solitary species in the table are large and powerful, their lethalities are low, resulting in generally low lethal

Table III

Lethal Capacity of Venoms of Ants and other Hymenoptera[a]

Species	Venom LD_{50} (mg/kg)	μg Venom/ Individual	Colony size	Lethal capacity (number of stings for LD_{50} dose) 25-g Mammal	2-kg Mammal
Mutillidae					
Dasymutilla klugii	71	420	Solitary	4.2[b]	—[b]
Anthophoridae					
Diadasia r. rinconis	76[c]	32	Solitary	59[b]	—[b]
Pompilidae					
Pepsis formosa pattoni	65	2500	Solitary	.65	—[b]
Apidae					
Apis mellifera	3.5	50	Many thousands	1.8	140
Vespidae					
Vespula (Paravespula) pensylvanica	10.7	70	Thousands	3.8	310
Formicidae					
Pseudomyrmex mexicanus	8.0	16	Tens to hundreds	12.5	—[b]
Dinoponera grandis[d]	38	550	Tens to hundreds	1.7	—[b]
Paraponera clavata	6.0	180	Hundreds	.8	67
Eciton burchelli	10	60	Many thousands	4.2	330
Ectatomma quadridens	6.5	160	Hundreds	1.0	81
Pogonomyrmex maricopa	.15	25	Many thousands	.15	12

[a]Data from Schmidt *et al.*, 1980 and unpublished.

[b]Lethal capacity so low that lethality is not a likely threat from this species.

[c]J. O. Schmidt and S. L. Buchmann, unpublished.

[d]Previously called *D. gigantea* in Schmidt *et al.* (1980).

capacities. *Pepsis* is an exception because the venom quantity in one of these enormous individuals is so great that even a single individual could be potentially threatening to a small bird or mammal. All of the ants and other social species listed on the table have venoms with high lethal capacities. This is especially true of the species which live as large populous colonies. Many of these can pose a serious health threat to even large vertebrate predators via stinging *en masse*. The harvester ants (*Pogonomyrmex*) are an interesting case in point. I am aware of no mammal or bird that is a serious predator of harvester ants. Their only serious vertebrate predators are the horned lizards of the genus *Phrynosoma* (Iguanidae), which are specialists, often feeding almost exclusively on harvester ants (Rissing, 1981; Knowlton, 1938). *Phrynosoma douglassi* is also approximately 200 times more resistant to the lethal effects of *Pogonomyrmex maricopa* venom than are mice. This suggests either a coevolutionary race between predator and prey or a generally higher resistance of lizards than mice to the venom (P. J. Schmidt and J. O. Schmidt, unpublished).

Although the data in Table III are based on the assumptions that an insect can deliver all of its venom, that venom activity is equal among different vertebrate species, and that venom activity is equal by different routes of injection, they do indicate that venom effectiveness against vertebrate predators can be improved for species with populous colonies via increased toxicity. This is made more plausible by the fact that some individual predators in a population are likely to be more sensitive than others, and thereby, could be more easily damaged.

Venom pain and toxicity should not be viewed as isolated properties; often the two synergize each other. The pain induced by a sting serves as an immediate deterrent and as a stimulus which later can be associated with the toxic and damaging effects of envenomation. A toxic activity of a venom can serve as the actual punishment to reduce the likelihood that the predator can view the pain as a form of harmless (albeit unpleasant) Batesian mimicry.

One final group of components in ant venoms further synergizes venom effectiveness against vertebrate predators. These are the venom-produced alarm and recruitment pheromones that mediate the behaviors so crucial for effective mass attack and envenomation. More about these venom components will be presented later.

C. Chemical Communication

The final, but by no means least important, biological role of ant venoms is chemical communication. Effective behavioral interaction among members of an ant colony is controlled by a variety of pheromones. These pheromones

are produced in an assortment of locations in the body of the ants including the mandibular glands, the Dufour's glands, the pygidal (anal) and other abdominal glands, the metapleural glands, the epicuticular surface, and the venom glands. Venom glands in ants are frequently sources of alarm pheromones, trail pheromones, sex pheromones, attractant-recruitment pheromones, aggregation pheromones, and recognition pheromones. The known chemistry and activities of these venom-produced pheromones will be discussed in later sections of this chapter.

IV. ANT VENOM COLLECTION AND PURITY

Few subjects could be more esoteric and less interesting than the collection of ant venoms. Nevertheless, obtaining ant venoms in quantity is the major impediment to studies involving these interesting and little-understood venoms and the purity of the collected venoms is absolutely crucial for qualitative and quantitative investigation. Many of the problems in the detection and quantitation of activities (e.g., LD_{50}, enzymes), not to mention comparisons with other venoms, arise from the use of impure venoms as starting materials. Impure venoms contain nonvenom contaminants that, in turn, make accurate determination of activity per milligram venom impossible (see also Chapter 3). The contaminants may contain materials that could give false positives for enzyme assays and they may also complex with, hydrolyze, or otherwise interfere with venom components (e.g., Hoffman and Wood, 1984; King *et al.*, 1983).

Investigators should be wary of results reporting (especially low-level) enzyme activities in extracts of venom sacs, sting apparatuses, etc., unless pure venom was also demonstrated to have that activity in relevant and meaningful levels.

Ants as venom sources present two problems, getting enough ants and getting the venom out of the ants. Unlike honeybees, which can be electrically stimulated to obtain their venom, or social wasps, which, with proper handling, will extrude their venom through the sting tip, ants will not readily release venom by any means (see also Chapter 3). Electrical stimulation has been reported as a means to collect venoms from *Pogonomyrmex* and *Myrmica*, but these methods usually are not particularly effective or optimal because the ants do not readily release their venom and they release fluids through the mouth and anus plus glandular products when stimulated. Also, I knew of no confirmed case in which ants effectively sting through thin membranes. Fire ant venom can be painstakingly collected from the sting tip (Blum *et al.*, 1958), but this method of collection appears to be the exception rather than the rule.

For initial characterization of ant venoms, dissection is the method of choice. With a little practice, the venom reservoir (Fig. 4) can be pulled either with the sting shaft, or with the terminal sternite, out of the ant's gaster and placed in a droplet of distilled, deionized water. At this point, finely sharpened forceps are used to expose the reservoir from the rest of the tissue and to grasp the venom reservoir duct near where it enters the sting base. The duct is pinched off and the reservoir removed and placed in a clean droplet of water. All fat, hemolymph, etc., is now removed from the reservoir and/or forceps (transfer to a third droplet of water is sometimes necessary). The reservoir is then held by the end of the duct, pulled out of the water such that it hangs below the tips of the forceps, and is just touched to the surface of a final droplet of water used for temporary storage. At this stage, the forceps can be slightly and carefully opened, allowing the surface tension of the water to pull down the reservoir yet leave behind the (possibly contaminated) water that is held by capillary action between the tines of the forceps. Dirty forceps are wiped clean by pushing through clean tissue. When enough reservoirs are collected, each is torn with forceps and the venom allowed to drain. The empty reservoirs are then removed and discarded. Venom collected by these methods is essentially 100% pure. Nevertheless, if an activity of very low level is observed in the venom, it is always wise to assay a homogenate of ant gasters from which the venom apparatus was cleanly removed. This way the activity from general tissues in the gaster can be determined and compared to that of the venom.

Once these venom collection methods have been used to confirm venom activity and its quantitation, then gross extraction methods are in order to obtain quantities for isolation and purification (see also Chapter 3).

V. VENOM BIOCHEMISTRY AND CHEMISTRY

Ant venoms can be broadly categorized as either predominantly proteinaceous or as nonproteinaceous mixtures of simple organic molecules. Species of ants in groups that are considered to be ancestral produce venoms composed almost entirely of proteins, while many, but not all, highly derived species produce venoms that are rich in volatile organic compounds and low in proteins, and a great many species produce mainly proteinaceous venoms that have traces of highly active organic molecules that act as pheromones or allomones. This discussion will start with an in-depth treatment of the smaller molecules and then progress to the chemically more complex protein-based venoms.

A. Predominantly Nonproteinaceous Venoms

1. Formicine Ant Venoms

The first venom to be chemically described was that of an ant. In the seventeenth century Wray (1670) reported the distillation of formic acid from the formicine ant *Formica rufa* (see also Chapter 1). Since that time formic acid has been found in virtually all the species of ants in the subfamily Formicinae that have been studied (Stumper, 1922, 1951, 1952; Osman and Brander, 1961; Otto, 1060; Regnier and Wilson, 1968; Schreuder and Brand, 1972; Bradshaw *et al.*, 1975; Hefetz and Orion, 1982). The ubiquity of formic acid in the Formicinae and its apparent absence from the venoms of species in other subfamilies has led to the use of its presence as a diagnostic subfamilial character of the formicine ants. The venom of these ants may constitute up to 22% of the total weight of the ant, with formic acid constituting as much as 65% or more of the venom (Osman and Brander, 1961).

Although formicine venoms have been known to contain formic acid for more than 300 years, the mechanism of the synthesis of formic acid has only recently been investigated. The synthesis is intimately associated with one carbon (C-1) metabolism of the glandular cells of the poison glands. Studies with labeled precursors show that serine, glycine, and histidine may all contribute carbon atoms to the synthesis of formic acid (Hefetz and Blum, 1978a,b). Formic acid synthesis *in vivo* probably starts from serine, with the incorporation of the α and β carbons (but not the carboxyl carbon) into 5,10-methylene tetrahydrofolate (methenyl H_4F). This intermediate is then reduced to 10-formyl tetrahydrofolate (formyl H_4F), which is in turn hydrolyzed to produce formic acid and tetrahydrofolate (H_4F) (Fig. 5). The cells of the venom reservoir are rich in all of the enzymes required for these conversions. The pathway is apparently identical in the two species of *Camponotus* and three species of *Formica* that have been examined (Hefetz and Blum, 1978b). The toxic effects of formic acid on the secretory cells are apparently avoided through a 20:1 equilibrium at the last step, the conversion of formyl H_4F to formic acid, a step that probably consumes ATP. An energy-requiring carrier system appears to transport formic acid across the membrane of the glandular cells and into the glandular lumen. The system is self-regulating; the poison gland contents saturate the formic acid carrier system causing formic acid and ATP to accumulate in the glandular cells. The accumulation of these two materials inhibits the formation of formic acid from formyl H_4F. When the ant ejects formic acid from the reservoir,

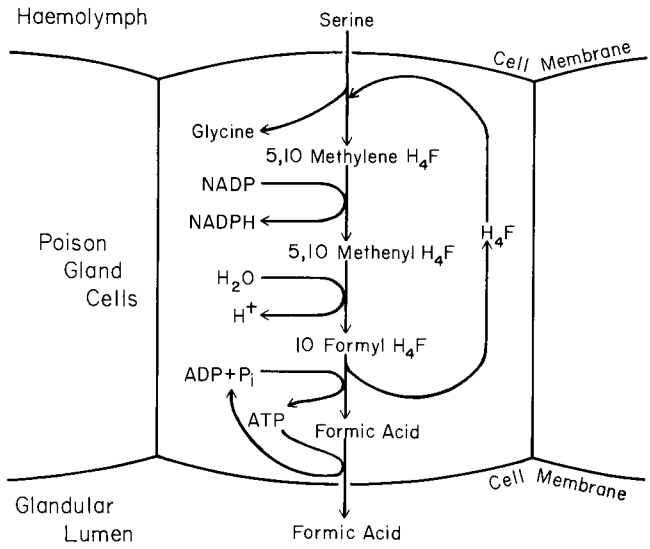


Fig. 5 Diagram of the biosynthesis of formic acid in the poison gland of formicine ants. After Hefetz and Blum (1978a,b).

the whole system is reactivated until the glandular reservoir is filled again (Hefetz and Blum, 1978b).

Other than formic acid, the only compounds in the venoms of formicine ants are amino acids and small peptides that constitute up to 5% of the venom (Osman and Brander, 1961; Hermann and Blum, 1968). Analyses of the venoms of four species, *Formica polyctena*, *F. rufa*, *F. nigricans*, and *F. pratensis*, revealed from 0.05 to 0.82% free amino acids and from 1.17 to 1.82% (by weight) peptides in fresh venom. Three peptides are distinguishable by electrophoresis and both the peptides and free amino acids contain high proportions of the acidic amino acids, glutamic and aspartic acids, and almost none of the basic amino acids (Osman and Brander, 1961).

Notwithstanding its simple chemistry, formic acid is a major part of the *élan vital* of formicine ants. It functions as the prime defense against predators, the crucial factor in interspecific competition with other formicids, and a main component of the chemical communication system. The cytotoxic and corrosive properties of formic acid, the most acidic of the simple aliphatic organic acids ($pK_a = 3.75$), make it a potent defense against vertebrate predators (Otto, 1960; Eisner *et al.*, 1972). Against arthropods, the action of formic acid is enhanced by admixture with the lipophilic contents of the Dufour's gland. The Dufour's gland hydrocarbons and other lipid soluble molecules aid by 'wetting' the arthropod waxy epicuticle and thereby assisting

the spreading and penetration of formic acid (Ghent, 1961; Regnier and Wilson, 1968). Formic acid vapors are also insecticidal (Osman and Kloft, 1961).

The formicine venom–Dufour's spray is exceedingly effective in battles with other ant species. The sprayed opponents usually retreat rapidly, often succumbing as a result of the toxic action (Bhatkar *et al.*, 1972). Part of the success of formicine ants [as well as dolichoderines which can spray pygidial (anal) gland products] in competition with stinging ants lies in this ability to spray from a distance an effective cuticle-penetrating liquid. Unlike the sting, which must penetrate the often hard cuticle of an adversary, formic acid–rich venom is effective when sprayed from a distance of several centimeters (Ghent, 1961). The possession of such weapons allows selection for quick, agile, thin-cuticled biting ants that can outmaneuver, and therefore outcompete, sluggish, heavily armored stinging ants such as ponerines (which must often battle for some time before effectively utilizing the sting).

In formicine and dolichoderine ants the mandibles and their glands have taken on new functions, possibly as a result of the possession of spray-based abdominal defenses. Formic acid penetration through arthropod cuticle is vastly improved by prior mandibular abrasion (Ghent, 1961). Thus, mandibles are often employed in strike-and-run attacks, during which time the venom may be brought into play. Also during the biting process, the mandibular glands of many formicines, including *Lasius, Acanthomyops* and probably many others, secrete terpenes and other volatile organic compounds that also greatly potentiate the penetration of formic acid through the opponent's cuticle (Ghent, 1961; Regnier and Wilson, 1968; Löfqvist, 1977). The combined synergism of the Dufour's and mandibular gland secretions with the venom is clearly demonstrated in the superiority of *Lasius neoniger* over *Solenopsis invicta*, a notably pugnacious species of stinging ant (Bhatkar *et al.*, 1972). It is noteworthy that the effectiveness of the fire ants in these encounters is increased greatly because they too possess a venom that when dabbed from the sting tip onto other insects is insecticidal (Blum *et al.*, 1958).

In addition to its defensive roles, formic acid plays a major role in the chemical communication of formicine ants. In itself the acid is a strong alarm pheromone for most formicine genera (Maschwitz, 1964; Löfqvist, 1976; Bradshaw *et al.*, 1975; Hefetz and Orion, 1982). Exceptions to this include *Lasius* (Maschwitz, 1964; Regnier and Wilson, 1969), *Plagiolepis* (Maschwitz, 1964) and *Acanthomyops* (Regnier and Wilson, 1968). The threshold concentration of formic acid for alarm behavior in *Formica rufa* is 7×10^{15} molecules/cm^3, or about 280 ppm (my calculations) (Löfqvist, 1976). Alarm response is also evoked by Dufour's gland products, especially undecane (Regnier and Wilson, 1968, 1969; Dumpert, 1972; Bradshaw *et al.*, 1975; Löfqvist, 1976). In *F. rufa* undecane releases alarm at a concentration of

5×10^{13} molecules/cm^3, or at a concentration ~100 times lower than formic acid (Löfqvist, 1976). *Lasius alienus* is exquisitely sensitive to undecane; alarm is elicited on average with 10^9 molecules/cm^3, a concentration about 10^7 less than the detection limit of *F. rufa* for formic acid (Regnier and Wilson, 1969).

For many formicine species undecane and formic acid act to synergize alarm behavior (Ayre and Blum, 1971; Löfqvist, 1976). Some formicines have also added mandibular gland components to the alarm pheromone arsenal (Bradshaw *et al.*, 1975; Hefetz and Orion, 1982). The communicative effects of formic acid and Dufour's or mandibular gland secretion are not simply additive or multiplicative; instead, they probably complement each other's activity via their own subtle roles. For example, Hefetz and Orion (1982) determined that formic acid is the main releaser of alarm and foraging aggressiveness in the 12 species studied, whereas the Dufour's gland products played roles varying from alarm to attraction and recruitment. The responses appeared to correlate with foraging behavior.

The actual formicid communicative fine tuning possible via combinations of venom, Dufour's, and mandibular glandular components has hardly been explored. Needless to say, this wealth of communicative possibilities including the venom contribution have allowed, in part, the development of the complex social behaviors and organizations that are the keys to the successful exploitation of virtually all the world's habitats by ants, including many formicines.

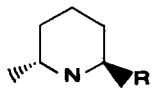
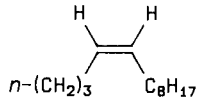
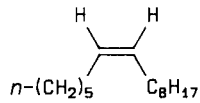
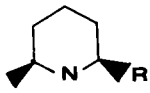
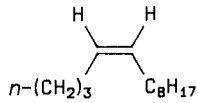
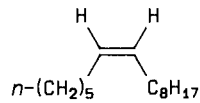
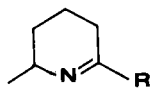
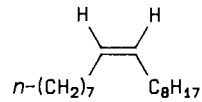
2. *Fire Ants [Solenopsis (Solenopsis) spp.]*

The large worldwide genus *Solenopsis* consists of numerous species of fire ants in the subgenus *Solenopsis* plus the much smaller thief ants in the subgenus *Diplorhoptrum* plus others (e.g., *Euophthalma*). Of these, only the fire ants of the subgenus *Solenopsis* are noted for their potent and effective stinging abilities. Although in the United States only a handful of fire ant species are present, in Latin America there are numerous, including many undescribed, species. At least three species, *S. invicta*, *S. richteri*, and *S. geminata*, have become established outside their native ranges and now are considered serious pests to humans and agriculture in the Carribean, southeastern United States, and in the islands in the Pacific Ocean (Réaumur, 1926; Buren *et al.*, 1974; Weber, 1950). Fire ants are not yet found in Europe or other Old World regions.

The venoms of *Solenopsis* are strikingly different from the protein-based venoms of the majority of stinging ants and other aculeate Hymenoptera. The venoms of the fire ants contain only traces (0.1–1%) of protein (Baer *et al.*, 1979; B. R. Paull, personal communication); the majority of the venom

Table IV

Piperidines and a Piperideine in the Venoms of the Fire Ants, *Solenopsis* (*Solenopsis*) spp.

Structure	Substituent (R)	Abbreviation
	n-C_7H_{15}	*cis*-C_7
	n-C_9H_{19}	*cis*-C_9
	n-$C_{11}H_{23}$	*cis*-C_{11}
	n-$C_{13}H_{27}$	*cis*-C_{13}
		*cis*-$C_{13:1}$
cis-2-Methyl-6-n-alkyl-piperidines	n-$C_{15}H_{31}$	*cis*-C_{15}
		*cis*-$C_{15:1}$
	n-C_7H_{15}	*trans*-C_7
	n-C_9H_{19}	*trans*-C_9
	n-$C_{11}H_{23}$	*trans*-C_{11}
	n-$C_{11}H_{21}$	*trans*-$C_{11:1}$
	n-$C_{13}H_{27}$	*trans*-C_{13}
		*trans*-$C_{13:1}$
trans-2-Methyl-6-n-alkyl-piperidines	n-$C_{15}H_{31}$	*trans*-C_{15}
		*trans*-$C_{15:1}$
		*trans*-$C_{17:1}$
2-Methyl-6-n-undecyl-$\Delta^{1,2}$-piperideine	n-$C_{11}H_{23}$	—

consists of alkaloids in which small aqueous droplets are suspended. All but one of the presently known fire ant alkaloids are methyl-n-alkylpiperidines. The identified alkaloids plus a simple system of abbreviations to replace the long chemical names are listed in Table IV. In the abbreviations, cis or trans refer to the conformation of the alkyl groups on the ring, C_n refers to the number of carbon atoms in the alkyl side chain, and, if the side chain is unsaturated, the unsaturation is indicated as $C_{n:1}$ [e.g., *trans*-$C_{13:1}$ = *trans*-2-methyl-6-(*cis*-4′-*n*-tridecenyl) piperidine]. All future designations will be abbreviations (Table IV).

Chemically, the venoms of the fire ants exhibit remarkable faithfulness to the basic 2-methylpiperidine skeleton, with only the ring geometry and

the length and unsaturation of the side chain varying. The one exception is 2-methyl-6-*n*-undecyl-$\Delta^{1,2}$-piperideine, a ring-unsaturated analog of C_{11} that is present in small quantities in the venom of *Solenopsis xyloni*. This compound was speculated to represent a biochemical precursor of the other venom alkaloids (Brand *et al.*, 1972). The alkyl side chains of venom piperidines vary in length from seven to 17 carbons with no chains of even numbers of carbons (Table IV). The most frequently identified piperidines possess 11, 13, and 15 carbon side chains (see Table VI). Those with longer side chains (13, 15, and 17 carbons) frequently contain a cis unsaturation nine carbons from the end. Although the methyl and alkyl substituents to the piperidine have been found to be either of the cis or trans geometry, the exact symmetry at the enantiomeric centers is not known.

The blends of alkaloids in the analyzed fire ant venoms are species specific. The most commonly found alkaloids are *trans*- and *cis*-C_{11}, followed by *trans*- and *cis*-$C_{13:1}$, and *trans*-$C_{15:1}$ (Table V). In terms of the relative quantities, *trans*-$C_{13:1}$ dominates in seven species, *cis*-C_{11} in three species, and *trans*-$C_{15:1}$, *trans*-C_{11}, and *trans*-C_9 in one species each. Above 11 carbons in chain length, the trans ring configuration predominates; at 11 carbons, cis is relatively more abundant. *cis*-C_{11} dominates the venoms of two endemic North American species, *Solenopsis xyloni* and *S. aurea*, plus the introduced species, *S. geminata*. Piperidines with long or short side chains are rare in fire ant venoms: a 17-carbon side chain is only known from the traces of $C_{17:1}$ (apparently *trans*) in worker venoms of *S. invicta* (Jones and Blum, 1983); C_9 is only known for the unnamed South American species #5, in which *trans*- and *cis*-C_9 are the only piperidines present (MacConnell *et al.*, 1976); and *cis*- and *trans*-C_7 have only been detected as minute traces in the venoms of female alates of *S. richteri* (Table V).

Overall, the blends of alkaloids in fire ant venoms are not especially chemically diverse. In these venoms only two to five constituents are present in quantities greater than 1% of the total. This is not, however, to imply that fire ant venoms are simple in their pharmacological activities, as will be discussed later.

In fire ants, the workers are the main individuals that use their venoms for defense or offense. The queens, other than brief times during the mating flights, virtually never use their venoms for defense (or offense), and even during mating flights, the defensive usefulness of the venom is questionable. As a consequence, response to selection pressure would be expected to be expressed most strongly in the alkaloidal compositions of the venoms of the workers (only one investigated species of fire ant, *Solenopsis geminata*, has large workers called 'majors'). When the venoms of fire ant queens (female alates) and majors are compared to those of workers, some interesting

Table V

Piperidine Alkaloids in the Venoms of Fire Ant Workers

Species of *Solenopsis*	Cis (quantity [a])						Trans (quantity[a])								Reference
	C_9	C_{11}	C_{13}	$C_{13:1}$	C_{15}	$C_{15:1}$	C_9	C_{11}	$C_{11:1}$	C_{13}	$C_{13:1}$	C_{15}	$C_{15:1}$	$C_{17:1}$	
Species present in North America															
S. aurea	0	++++	t	0	0	0	0	++	0	0	0	0	0	0	Blum *et al.* (1973)
S. xyloni	0	++++	+	+	0	0	0	+++	0	0	0	0	0	0	Brand *et al.* (1972)
S. geminata	0	+++	+	+	0	0	0	+++	0	0	0	0	0	0	Brand *et al.* (1972)
S. richteri	0	t	t	+	0	0	0	++	0	+	+++	0	+	0	Brand *et al.* (1972)
S. invicta	0	t	t	t	t	t	0	++	0	++	++	++	+++	t[b]	Brand *et al.* (1972)
Species present in South America															
S. saevissima	0	0	t	t	0	0	0	+	0	+	++++	0	+	0	MacConnell *et al.* (1976)
S. edwardi	0	+	0	0	0	0	0	++++	0	0	0	0	0	0	MacConnell *et al.* (1976)
S. sp 1[c]	0	0-+	t	+-++	t	t	0	+	0	+	++++	0	+	0	MacConnell *et al.* (1976)
S. sp 2	0	0	0	t	0	0	0	+	t	0	++++	0	+	0	MacConnell *et al.* (1976)
S. sp 3	0	t	0	t	t	t	0	+	0	0	++++	0	+	0	MacConnell *et al.* (1976)

(continued)

Table V (*continued*)

Piperidine Alkaloids in the Venoms of Fire Ant Workers

Species of *Solenopsis*	Cis (quantity[a])						Trans (quantity[a])								Reference
	C_9	C_{11}	C_{13}	$C_{13:1}$	C_{15}	$C_{15:1}$	C_9	C_{11}	$C_{11:1}$	C_{13}	$C_{13:1}$	C_{15}	$C_{15:1}$	$C_{17:1}$	
S. sp 4	0	0	0	t	0	t	0	+	t	0	++++	0	+	0	MacConnell *et al.* (1976)
S. sp 5	++	0	0	0	0	0	++++	0	0	0	0	0	0	0	MacConnell *et al.* (1976)
S. sp 6	0	0	0	0	0	0	0	+	0	+	+++	+	++	0	MacConnell *et al.* (1976)

[a]Percentage of total alkaloids: ++++, >75%; +++, 36–75%; ++, 10–35%; +, 1–10%; t, <1%; and 0, not detectable.
[b]Jones and Blum (1983) reported $C_{17:1}$ as a trace.
[c]Four collections surveyed.

patterns emerge. In virtually all four cases *cis*- and *trans*-C_{11} dominated the alate venoms, almost to the exclusion of other components. In these venoms, the ratio of cis to trans always favored the cis (ratios of 1.86 to 17.6 to 1) Brand *et al.*, 1973a; MacConnell *et al.*, 1974). When compared to conspecific workers, the queens and majors had a 2.24–32.4 times higher ratio of *cis*-C_{11} to *trans*-C_{11} than the workers (Table VI).

The chemical findings, taken in conjunction with biological data and human reactions to stings, provide the basis for a plausible biochemical evolution of fire ant venom chemistry. Of the species present in North America, *Solenopsis invicta* is the most ecologically successful and is the species that induces the greatest pain in stung humans. *S. richteri* is also successful, but as a result of competition with *invicta*, its range is small. Its stings are essentially as painful as those of *invicta*. *Solenopsis geminata*, *S. xyloni*, and *S. aurea* all produce less painful stings and are all much less conspicuous in most environments. Furthermore, the stings of *S. invicta* and *S. richteri* produce necrotic pustules in human skin, whereas the stings from the other three species do not (Caro *et al.*, 1957). The venoms of *S. invicta* and *S. richteri* are both dominated by trans alkaloids with 13- and 15-carbon side chains; the venoms of the other three species are dominated with cis-alkaloids, especially the shorter *cis*-C_{11}. This led Brand *et al.* (1973b) to postulate that the longer-chained trans piperidines are responsible primarily for the greater pain and effectiveness against humans of the venoms of *S. invicta* and *S. richteri*. Since they are more effective against large predators, they probably are more evolutionarily derived than the shorter cis piperidines. The data from the queens supports this idea as well; queens would experience little selection pressure to develop more effective venoms and, hence, the compositions of their venoms are likely to remain ancestral, that is, rich in shorter-chained cis alkaloids (*cis*-C_{11}). Majors probably represent a caste hormonally more similar to the queens and are also constructed with primary emphasis not for fighting but for use of the mandibles as seed-crushing organs (R. K. Vander Meer, personal communication). Therefore, it is not surprising that their venom would appear more ancestral in nature than that of the minor workers.

The biological data on the South American species analyzed and represented in Table V are not available. Thus, comparisons of their venoms with their ecological success cannot easily be made. It can be noted that *Solenopsis saevissima* stings painfully and effectively, and its venom, like those of the *S. invicta* and *S. richteri*, contains mainly trans long-chained piperidines. *Solenopsis edwardi*, a close relative of *S. geminata*, produces mainly *trans*-C_{11}. Whether or not this observation represents a first step toward evolutionary development of more effective venoms can only be speculated.

Table VI

Comparison of Venom Alkaloidal Contents of Fire Ant Alate Queens and Majors with Workers[a]

Species of *Solenopsis*	Caste	*cis*-C_{11} / *trans*-C_{11}	Other alkaloids	*cis*-C_{11}: *trans*-C_{11} of alates or majors / *cis*-C_{11}: *trans*-C_{11} of workers
S. xyloni	Alates	17.6	None	4.43
	Workers	3.97	See Table V	—
S. geminata	Alates	3.36	0.2% *cis*-C_9	2.24
	'Majors'	4.94	None	3.29
	Workers	1.50	See Table V	—
S. richteri	Alates	1.86	1% *cis*-C_9, .8% *trans*-C_9, tiny traces *cis*- and *trans*-C_7	>18.6
	Workers	<.1	See Table V	—
S. invicta	Alates	3.24	None	>32.4
	Workers	<.1	See Table V	—

[a]Data from Brand *et al.* (1973a); MacConnell *et al.* (1974).

The whole evolutionary scenario postulated by Brand *et al.* (1973b), though appealing, needs much further testing. For example, the effectiveness of the various alkaloids against invertebrate predators and competitors needs investigation, as does the effectiveness of the venoms of the South American species against humans (the sting reactions of species 5 would be especially interesting).

The aqueous portion of fire ant venom has been an extremely intractable material to investigate. Early investigations using butanol extracted pure venom of *Solenopsis invicta* revealed three small cathode-migrating bands plus two fainter bands on electrophoresis. Seven amino acid residues were also demonstrated (Buffkin and Russell, 1974). Baer *et al.* (1977, 1979) recovered 0.1% protein from samples of pure *S. invicta* venom that had been extracted with hexane to remove the alkaloids. Gel filtrations of this material on Sephadex G-50 resolved three protein bands with minimum molecular weights of 10,000, 5000 and 2000. The largest protein exhibited phospholipase A (PLA), as well as another (probably phospholipase B) activity. Hyaluronidase was detected in the whole venom, though due to the small quantities of material available, its activity could not be related to any of the isolated electrophoretic bands. In a preliminary abstract, another team of investigators reported the isolation by Sephadex gel filtration of 21 proteins from pure venom of *S. invicta*. The molecular weights of the proteins ranged from 5000 to 82,000, and these proteins plus four others constituted the proteins isolated from whole body extracts of the ants (Paull *et al.*, 1984). It will be interesting to see the full report on this work.

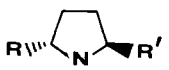
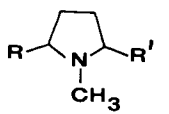
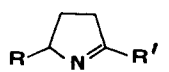
3. *Other Subgenera of Solenopsis: Diplorhoptrum and Euophthalma*

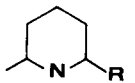
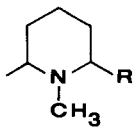
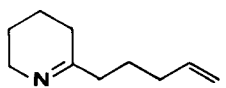
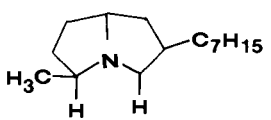
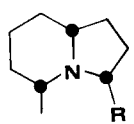
Unlike the fire ants, thief ants (*Diplorhoptrum*) and *Solenopsis* (*Euophthalma*) spp. are small to tiny and are retiring in habits. These ants are noted for their ability to enter through minute tunnels into the brood chambers of fire ants and other ant species. Here they repel the attending host workers by releasing their venom and then steal the hosts' brood, which is carried off for food (Hölldobler, 1928). These diminutive ants are unaggressive toward humans or large animals and their stings are essentially ineffective on people (Pedder *et al.*, 1976).

Species in the subgenera *Diplorhoptrum* and *Euophthalma* produce the greatest diversity of venom alkaloids of any ant groups. They produce a variety of *trans*-2,5-dialkylpyrrolidines with saturated alkyl side chains of two, four, five, six, or seven carbons in length; 2,5-dialkylpyrrolines with saturated side chains of two, five, and seven carbons; and *N*-methyl-2-butyl-5-pentylpyrroline (Table VII). The synthesis of these five-membered

Table VII

Venom Components of Thief ants [*Solenopsis* (*Diplorhoptrum*)] and *Solenopsis* (*Euophthalma*) *littoralis*

Venom constituent	Substituent R	Substituent R′	Species of *Solenopsis*	Reference
	C_2H_5	C_7H_{15}	*S. punctaticeps*	Pedder *et al.* (1976)
	n-C_4H_9	n-C_5H_{11}	*S. punctaticeps*	Pedder *et al.* (1976)
	n-C_4H_9	n-C_7H_{15}	*S. punctaticeps*	Pedder *et al.* (1976)
	n-C_4H_9	n-C_7H_{15}	*S. fugax*	Blum *et al.* (1980)
	n-C_5H_{11}	n-C_6H_{13}	*S. texana*	Jones *et al.* (1979)
trans-2,5-Dialkylpyrrolidines	n-C_5H_{11}	n-C_6H_{13}	*S. molesta*	Jones *et al.* (1979)
	n-C_4H_9	n-C_5H_{11}	*S. punctaticeps*	Pedder *et al.* (1976)
N-Methyl-2-butyl-5-pentylpyrrolidine				
	C_2H_5	n-C_5H_{11}		
	n-C_5H_{11}	C_2H_5	*S. punctaticeps*	Pedder *et al.* (1976)
	C_2H_5	n-C_7H_{15}		
2,5-Dialkylpyrrolines	n-C_7H_{15}	C_2H_5		
	n-C_9H_{19} (cis)	—	*S. conjurata*	*Jones et al.* (1984)
	(trans)	—	*S. conjurata*	
	(trans)	—	*S. carolinensis*	
	(unknown)	—	*S.* sp B	Jones *et al.* (1982a)
	n-$C_{11}H_{23}$ (trans)	—	*S. pergandei*	
	(cis)	—	*S. conjurata*	Jones *et al.* (1984)

 2-Methyl-6-alkylpiperidines	n-$C_{13}H_{27}$ (unknown)	—	*S. littoralis*	Jones *et al.* (1982a)
 *N*-Methyl-2-methyl-6-alkylpiperidines	n-C_9H_{19} (trans) (cis) (unknown)	— — —	*S. conjurata* *S. conjurata* *S. littoralis*	Jones *et al.* (1984)
	n-$C_{11}H_{23}$ (unknown)	—	*S. pergandei*	Jones *et al.* (1982a)
 2-(4-Penten-1-yl)-1-piperideine	—	—	*S.* sp. A	Jones *et al.* (1982a)
 (5*Z*,8*E*)-3-Heptyl-5-Methylpyrrolizidine	—	—	*S.* aff. *tennesseensis*	*Jones et al.* (1980a)
 (5*Z*,9*Z*)-3-Alkyl-5-methylindolizidine	C_2H_5 n-C_6H_{11}	— —	*S. conjurata* *S.* sp. AA (queens)	Jones *et al.* (1984)

ring compounds with side chains of intermediate length represents a major chemical difference between these ants and the fire ants. To date, the latter have been found to produce only six-membered rings with a methyl and a long-chained alkyl substituent. The differences between the two venoms appear functional as well as structural: the venom secretions of the thief ants were found to be highly repellent to host workers (Hölldobler, 1973), whereas piperidines exhibit a variety of offensive and defensive properties but are not highly repellent. Pure *trans*-2-butyl-5-heptylpyrrolidine in quantities equivalent to those produced by *Solenopsis fugax* was found to be as repellent to workers from six different genera of ants as the whole venom. Moreover, insect pieces or host brood when dabbed with the pyrrolidine repelled ants for up to an hour (Blum *et al.*, 1980).

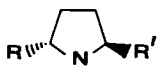
In addition to producing five-membered nitrogen-containing rings, thief ants and *Solenopsis* (*Euophthalma*) *littoralis* synthesize piperidines similar to those found in fire ant venoms (Table VIII). The side chains contained 9, 11, or 13 carbons. In that regard they are identical to some of the constituents in fire ant venoms. In addition to these compounds, thief ants have also been discovered to contain *N*-methylpiperidines, 2-(4-penten-1-yl)piperidine, and both (5*Z*,9*Z*)-3-alkyl-5-methyindolizidines, all compounds not present in fire ants. *Solenopsis tennessensis* is yet one step more complicated in its synthetic repertoire, producing the bicyclic pyrrolizidine, (5*Z*,8*E*)-3-heptyl-5-methylpyrrolizidine (Jones *et al.*, 1980a). The biological role of this compound, like that of many of the previously described, awaits analysis. It would not be surprising, however, if most of these compounds contribute either directly as major components responsible for venom activity or as synergists to the activities of other components.

4. *Venoms of Monomorium*

This group of diminutive ants, like the thief ants, often plunders brood from other species of ants (Hölldobler, 1973). Unlike thief ants, however, *Monomorium* often forages above ground, where it is notably successful at stealing prey from other species of ants (Baroni Urbani and Kannowski, 1974; Hölldobler, 1973; Adams and Traniello, 1981). Both brood and prey theft are accomplished by use of venom discharge. The venom effectively repells foragers from their prey or from their immobile brood. *Monomorium* species have specialized in the production of venoms containing five-membered nitrogenous ring compounds, including nine different dialkylpyrrolidines, three *N*-methyl-dialkylpyrrolidines, and four dialkylpyrrolines (Table VII). Although these natural products at first appear to be the same as those of

Table VIII

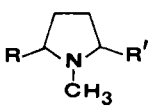
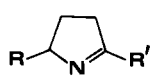
Venom Components of *Monomorium*

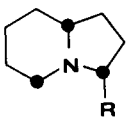
Venom constituent	Substituent R	Substituent R′	Species of *Monomorium*	Reference
 2,5-Dialkylpyrrolidines	n-C_4H_9	n-C_5H_{11}	*M. pharaonis*	Talman *et al.*, 1974; Jones *et al.*, 1984
	n-C_4H_9	n-C_5H_{11}	*M. latinode*	Jones *et al.*, 1982a
	n-C_4H_9	n-C_5H_{11}	*M. subopacum*	
	n-C_4H_9	n-C_7H_{15}	*M. latinode*	
	n-C_4H_9	n-C_7H_{15}	*M. subopacum*	
	n-C_6H_{13}	n-C_9H_{19}	*M. viridum*	Jones *et al.*, 1982b
	n-C_6H_{13}	n-C_9H_{19}	*M. floricola*	
	n-C_6H_{13}	n-C_9H_{19}	*M. ebeninum*	
	n-C_6H_{13}	n-C_9H_{19}	*M.* near *metoecus*	
	n-C_5H_{11}	n-$(CH_2)_4CH=CH_2$	*M. pharaonis*	Ritter *et al.*, 1975
	n-C_7H_{15}	n-$(CH_2)_3CH=CH_2$	*M. subopacum*	Jones *et al.*, 1982a
	n-C_7H_{15}	n-$(CH_2)_4CH=CH_2$	*M. pharaonis*	Ritter *et al.*, 1975
	n-C_9H_{19}	n-$(CH_2)_3CH=CH_2$	*M. minimum*	Jones *et al.*, 1980b
	n-C_9H_{19}	n-$(CH_2)_3CH=CH_2$	*M. viridum*	
	n-C_9H_{19}	n-$(CH_2)_3CH=CH_2$	*M. pharaonis*	Ritter *et al.*, 1975
	n-C_9H_{19}	n-$(CH_2)_4CH=CH_2$	*M. viridum*	Jones *et al.*, 1982b
	n-C_9H_{19}	n-$(CH_2)_4CH=CH_2$	*M. cyaneum*	
	n-C_9H_{19}	n-$(CH_2)_4CH=CH_2$	*M. ebeninum*	
	n-C_9H_{19}	n-$(CH_2)_4CH=CH_2$	*M.* near *minimum*	
	n-C_9H_{19}	n-$(CH_2)_4CH=CH_2$	*M. minutum*	

(continued)

Table VIII (*continued*)

Venom Components of *Monomorium*

Venom constituent	Substituent R	Substituent R′	Species of *Monomorium*	Reference
2,5-Dialkylpyrrolidines, continued	n-C_9H_{19}	n-$(CH_2)_4CH=CH_2$	*M.* near *emersoni*	*Jones et al.*, 1982b
	n-C_9H_{19}	n-$(CH_2)_4CH=CH_2$	*M.* near *metoecus*	
	n-$(CH_2)_4CH=CH_2$	n-$(CH_2)_7CH=CH_2$	*M. viridum*	
	n-$(CH_2)_4CH=CH_2$	n-$(CH_2)_7CH=CH_2$	*M. minimum*	
	n-$(CH_2)_4CH=CH_2$	n-$(CH_2)_7CH=CH_2$	*M.* near *minimum*	
	n-$(CH_2)_4CH=CH_2$	n-$(CH_2)_7CH=CH_2$	*M.* near *metoecus*	
	n-$(CH_2)_4CH=CH_2$	n-$(CH_2)_7CH=CH_2$	*M.* near *emersoni*	
	n-$(CH_2)_4CH=CH_2$	n-$(CH_2)_7CH=CH_2$	*M. ebeninum*	
	n-$(CH_2)_4CH=CH_2$	n-$(CH_2)_7CH=CH_2$	*M. minutum*	
	n-$(CH_2)_4CH=CH_2$	n-$(CH_2)_7CH=CH_2$	*M. carbonarium*	
 *N*-Methyl-2,5-dialkylpyrrolidines	n-C_4H_9	n-C_7H_{15}	*M. latinode*	Jones *et al.*, 1982a
	n-$(CH_2)_4CH=CH_2$	n-$(CH_2)_7CH=CH_2$	*M. viridum*	Jones *et al.*, 1982b
	n-$(CH_2)_4CH=CH_2$	n-$(CH_2)_7CH=CH_2$	*M. minimum*	
	n-$(CH_2)_4CH=CH_2$	n-C_9H_{19}	*M. viridum*	
	n-$(CH_2)_4CH=CH_2$	n-C_9H_{19}	*M.* near *emersoni*	
	n-$(CH_2)_4CH=CH_2$	n-C_9H_{19}	*M. cyaneum*	
	n-$(CH_2)_4CH=CH_2$	n-C_9H_{19}	*M.* near *metoecus*	
 2,5-Dialkylpyrrolines	n-C_4H_9	n-C_5H_{11}	*M. latinode*	Jones *et al.*, 1982a
	n-C_4H_9	n-C_7H_{15}	*M. latinode*	
	n-C_4H_9	n-C_7H_{15}	*M. subopacum*	
	n-$(CH_2)_4CH=CH_2$	n-$(CH_2)_7CH=CH_2$	*M. ebeninum*	Jones *et al.*, 1982b
	n-$(CH_2)_4CH=CH_2$	n-$(CH_2)_7CH=CH_2$	*M. viridum*	
	n-$(CH_2)_4CH=CH_2$	n-$(CH_2)_7CH=CH_2$	*M.* near *metoecus*	

	n-$(CH_2)_7CH=CH_2$	n-$(CH_2)_4CH=CH_2$	*M. ebeninum*	
	n-$(CH_2)_7CH=CH_2$	n-$(CH_2)_4CH=CH_2$	*M. viridum*	
	n-$(CH_2)_7CH=CH_2$	n-$(CH_2)_4CH=CH_2$	*M.* near *metoecus*	
	n-C_4H_9	—	*M. pharaonis*	Ritter *et al.*, 1973; Ritter and Persoons, 1975; Jones *et al.*, 1984
(5*Z*,9*Z*)-3-Alkyl-5-methylindolizidines	n-$(CH_2)_2CH=CHCH_2CH_3$	—	*M. pharaonis*	Ritter *et al.*, 1975

thief ants, in actual count the two taxa have in common only two compounds. *Monomorium* produces an additional 16 genus-specific compounds and *Solenopsis* (*Diplorhoptrum* and *Euophthalma*) 14 subgenus-specific compounds. The compounds in thief ants contain only saturated side chains that are short, two to seven carbons in length, whereas *Monomorium* produces a medley of both saturated alkyl side chains as well as many with terminal unsaturations. Also, the *Monomorium* derivatives have generally longer side chains (four to nine carbon length) than those of the diplorhoptrums.

Twelve of the 13 analyzed species of *Monomorium* possess venom with only five-membered nitrogen heterocyclic rings. The exception is the Pharaoh's ant, *M. pharaonis*, whose venom also contains (5*Z*,9*Z*)-3-butyl-5-methylindolizidine (all-*cis*-5-methyl-3- butyloctahydroindolizine) and 3-(5-hexen-1-yl)-5- methylindolizidine (Table VII) (Ritter *et al.*, 1973, 1975; Jones *et al.*, 1984). No piperidines such as found in the fire ants or thief ants have been detected in *Monomorium*. A variety of themes within the venoms of *Monomorium* are observed; some species such as *M. carbonarium*, *M. minutum*, and *M. minimum* contain only one, or one major, alkaloid whereas others such as *M. viridium* contain up to eight derivatives (Jones *et al.*, 1982b). One species, *M. floricola*, possesses very low levels of any venom alkaloids (Jones *et al.*, 1982b). The venoms of *Monomorium* also appear to be species specific: to date only two species, *M. minutum* and a species with affinities near *M. minimum* contain identical venom compounds, and even in this case the ratios of the two compounds are strikingly different (Jones *et al.*, 1982b).

Excluding use as a chemical defense to repell opponents and competitors, little is known of the biological role of the compounds in the venoms of *Monomorium*. The only species studied in biological detail is the Pharaoh's ant, a cosmopolitan tramp species that is noted as a nuisance and, via the transfer of microorganisms, as a health hazard in hospitals and homes. All-*cis*-5-methyl-3-butyloctahydroindolizine and *trans*-5-(hex-5-enyl)-2-pentylpyrrolidine were found to be attractant with some weak trail pheromonal activity (Ritter *et al.*, 1973; Edwards and Pinniger, 1978), the latter also induces queens to aggregate (Ritter *et al.*, 1977b). The true trail pheromone of Pharaoh's ant is produced by the Dufour's gland and is (6*E*,10*Z*)-2,3,7,11-tetramethyl-6,10-tridecadienal (Ritter *et al.*, 1977a). Although all the roles of the venom alkaloids, even in *M. pharaonis*, are not precisely quantified, the individual constituents clearly seem to exhibit parsimonious roles and frequently interact synergistically (Ritter and Stein, 1978).

5. *Trail Pheromones in Ant Venoms*

One of the preeminent factors in the ecological success of ants is their use of chemical trails. Chemical trails deposited by one individual can act to alert

other individuals, to provide a path to follow to some resource, and even to communicate information related to the richness of the resource. Some trail pheromones can also communicate that the trail is old and leads to a previously good foraging area that may be worth exploring in case no newer trails are present. Some components in trail pheromones may last only a few seconds or minutes as in the case of *Leptogenys chinensis* (Maschwitz and Schönegge, 1977) or they may last weeks, for example, *Atta* and *Pogonomyrmex* (Blum *et al.*, 1964; Hölldobler and Wilson, 1970). Trail-laying ability is a general character of ant species which have populous colonies.

Trail pheromones are produced by the hind gut, the Dufour's gland, and the venom gland as well as various specialized glands such as tarsal glands and various abdominal glands. The fact that trail pheromones have evolved repetitively and within a diversity of formicid glands demonstrates not only the polyphyletic origin of trail pheromones, but also the crucial importance of this ability to ants. The venom of ants is generally designed for offensive or defensive purposes. Nevertheless, venoms of many ant species have taken on parsimonious roles as sources for trail or sex pheromones (see next section). Table IX is a listing of ant taxa for which current evidence demonstrates the presence of trail pheromones in the venom. Of the 20 genera listed in the table, all except three ponerine genera are myrmicines. Since all trail pheromones must posses some volatility to function, they cannot be proteins or amino acids. The diversity of myrmicine species with venom-based trail pheromones thus demonstrates the widespread capability of members of this subfamily to synthesize and store volatile organic molecules in the venom gland and reservoir.

Trail pheromones are sometimes specific to species or to species group or to genus, but often extensive cross-sensitivity is observed. For example, *Pheidole militicida* will not follow trails of the sympatric species *P. tucsonica* or *P. rugulosa* (Hölldobler and Möglich, 1980), yet six species of *Myrmica* in one study followed trails generated from other species (Blum, 1974) and one of these species plus seven others also followed the same trail pheromone (Evershed *et al.*, 1982). Similar examples of intrageneric cross sensitivity are reported for *Novomessor* (Hölldobler *et al.*, 1978) and *Leptogenys* (Fletcher, 1971). Some species exhibit extensive cross-generic sensitivity of trail pheromones. Examples are found among the attine ants, among which species within with genera *Trachymyrmex*, *Acromyrmex*, *Atta*, and *Cyphomyrmex* follow each others' trails (Blum *et al.*, 1964; Robinson *et al.*, 1974) and among the tribe Myrmicini, *Manica*, *Myrmica*, and *Pogonomyrmex* will follow trails prepared from each others' venom gland extracts (Blum, 1974).

Chemical analyses of venom-based trail pheromones has helped explain some of the behavioral observations presented earlier. The first isolation and identification of a trail pheromone revealed the simple molecule methyl 4-methylpyrrole-3-carboxylate (Table X) present in 0.6-ng quantities per worker. This compound was the major component of the trail of *Atta texana*

Table IX

Species of Ants with Trail Pheromones in their Venom

Species	Reference
Subfamily Myrmicinae	
Acromyrmex (3 spp.)	Blum *et al.* (1964); Robinson *et al.* (1974)
Apterostigma collare	Robinson *et al.* (1974)
Atta (5 spp.)	Moser and Blum (1963); Blum *et al.* (1964); Robinson *et al.* (1974)
Cyphomyrmex rimosus	Blum *et al.* (1964)
Harpagoxenus americanus (tandem running)	Möglich (1979)
Huberia striata	Blum (1966)
Leptothorax (10 spp.) (tandem running)	Möglich (1979)
Manica (3 spp.)	Blum (1974)
Monomorium (3 spp.)	Blum (1966)
Myrmica (13 spp.)	Blum (1974); Evershed *et al.* (1982)
Novomessor (2 spp.)	Hölldobler *et al.* (1978)
Pheidole (5 spp.)	Hölldobler and Möglich (1980); Wilson (1976)
Pogonomyrmex (5 spp.)	Hölldobler and Wilson (1970); Hölldobler (1976a)
Sericomyrmex urichi	Blum and Portocarrero (1966)
Tetramorium (2 spp.)	Blum and Ross (1965)
Trachymyrmex (2 spp.)	Blum *et al.* (1964); Robinson *et al.* (1974)
Veromessor pergandei	Blum (1974)
Subfamily Ponerinae	
Leptogenys (3 spp.)	Fletcher (1971); Maschwitz and Schönegge (1983)
Megaponera foetens	Longhurst *et al.* (1979a)
Cerapachys turneri	Hölldobler (1982)

(Tumlinson *et al.*, 1971, 1972). Unlike ant alarm pheromones, which are often present in microgram or more quantities and are emitted as large short-lived vapor pulses, the methylpyrrole carboxylate is elaborated in minute quantities and is detactable by the ants in a trail as low as 80 fg/cm or 3.5×10^8 molecules/cm of trail (Tumlinson *et al.*, 1971). Riley *et al.* (1974) then identified the same compound from among other trace unidentified trail pheromone constituents in the venom of a sibling species, *A. cephalotes*. Higher quantities of the material, 1.3–13 pg/cm of trail, were required to induce trail following in *A. cephalotes* than in *A. texana*. The list of species whose venom contains methyl 4-methylpyrrole-2-carboxylate as the major component of the trail pheromone was further expanded to include a member from another attine genus, *Acromyrmex octospinosus*, which also followed trails of 12 pg/cm (Cross *et al.*, 1982). Earlier, Cross *et al.* (1979) had isolated the carboxylate in ~68 pg per ant quantities from the venom of *Atta sexdens rubropilosa* but had found the compound to be inactive as a trail pheromone.

In a second extraction of ants they discovered five times as much 3-ethyl-2,5-dimethylpyrazine, which produced good trail-following behavior at 0.2 to 20 pg/cm trail. This pyrazine was the only active compound discovered and appeared to be the sole trail pheromonal constituent of *A. sexdens* (Cross *et al.*, 1979). An opposite but analogous situation was discovered in the venom of the previously mentioned *Acromyrmex octospinosus*: this species produces 3-ethyl-2,5-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine as well as methyl 4-methylpyrrole 2-carboxylate. In this case the pyrazines are inactive and the pyrrole was solely responsible for the observed trail following activity (Cross *et al.*, 1982).

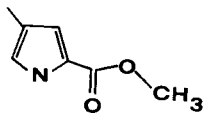
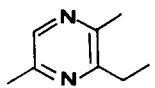
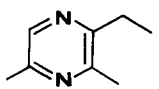
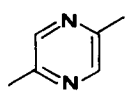
From the chemical analyses of these trail pheromones and earlier behavioral work by various authors, the picture emerges that only one, or a very few, compounds constitute the trail pheromone of a great many, if not most, of the fungus (attine) ants. Robinson *et al.* (1974) demonstrated the near ubiquity of methyl 4-methylpyrrole 2-carboxylate as the attine trail pheromone when they showed that 11 species in five attine genera all followed biologically meaningful levels of the synthetic compound. In this study they also demonstrated the uniqueness of *Atta sexdens* (esp. *A. s. rubropilosa*) in not following that trail substance. Not one non-attine species from 11 genera (five subfamilies) or the termite *Reticulitermes flavipes* would follow trails generated from the pyrrole carboxylate.

The chemistry of the trail pheromones of only one other ant tribe, the Myrmicini, have been investigated. As with the attines, only one or two nitrogenous ring compounds appear to be the active trail pheromones for many species in several genera. In the case of this tribe, subtituted pyrazines rather than a pyrrole derivative form the basis of the trail. 3-Ethyl-2,5-dimethylpyrazine (Table X), present in the venom of *Myrmica rubra* at a level of 5.8 ng per ant, was the only active trail compound (Evershed *et al.*, 1981). This compound, the same trail active compound found in the 'odd' attine *Atte sexdens rubropilosa*, is also the trail pheromone of all six additional Old World species of *Myrmica* tested (Evershed *et al.*, 1982) and in all likelihood of the five New World species that also followed trails of *M. rubra* (Blum, 1974). Moreover, this pyrazine is probably at least a constituent of the trail pheromones of *Manica* (three spp. tested) as well as *Pogonomyrmex* (two spp. tested) (Blum, 1974). Progress is currently underway to chemically confirm this in the case of *Pogonomyrmex* in our and other labs.

Tetramorium caespitum, another member of the Myrmicini in the broad sense (R. W. Taylor, personal communication), was discovered to contain 3-ethyl-2,5-dimethylpyrazine as a compound of its venom-based trail pheromone (Attygalle and Morgan, 1983). In this case, however, the species contained 2,5-dimethylpyrazine as well (total pyrazines of 3.9 ng per ant) and responded more actively to trails made from a 7:3 blend of the two than to either pure compound or to any other blend of the two.

Table X

Trail Pheromones from Ant Venoms

Venom constituent	Species	Reference
 Methyl 4-methylpyrrole-2-carboxylate	*Atta texana*	Tumlinson *et al.* (1971, 1972)
	Atta cephalotes	Riley *et al.* (1974)
	Atta sexdens rubropilosa[a]	Cross *et al.* (1979)
	Acromyrmex octospinosus	Cross *et al.* (1982)
 3-Ethyl-2,5-dimethylpyrazine	*Atta sexdens rubropilosa*	Cross *et al.* (1979)
	Myrmica rubra and eight others	Evershed *et al.* (1981, 1982)
	Tetramorium caespitum	Attygalle and Morgan (1983, 1984)
	Acromyrmex octospinosus[a]	Cross *et al.* (1982)
 2-Ethyl-3,5-dimethylpyrazine	*Acromyrmex octospinosus*[a]	Cross *et al.* (1982)
 2,5-Dimethylpyrazine	*Tetramorium caespitum*	Attygalle and Morgan (1983, 1984)

[a]Trail activity could not be demonstrated for this species.

To date, no chemical studies have identified venom-originating trail pheromones from the Ponerine subfamily or from the myrmicine tribes Pheidolini or Leptothoracini (tribes Crematogastrini and Solenopsidini appear to produce trail pheromones in the tarsal and Dufour's glands, respectively). It would be most interesting to see whether trail pheromones are generally conservative within these tribes, as appears for the two tribes studied, or whether their chemistry will vary within tribe in a similar fashion to the chemistry of alarm pheromones. In alarm pheromones, there is, at best, only a similarity among the compounds with tribes and genera. Such information, in addition to its practical aspects for potential control of undesired formicid species, may illuminate possible phylogenetic relationships. Indeed, the independent placement of *Tetramorium* in the tribe Myrmicini by R. W. Taylor (personal communication) is consistent with the finding that the trail pheromone from this tribe is also in the venom of *Tetramorium* (Attygalle and Morgan, 1983).

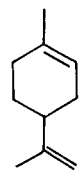
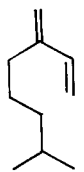
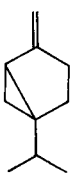
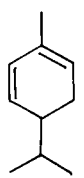
6. Other Pheromones and Miscellaneous Small Molecules in Ant Venoms

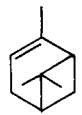
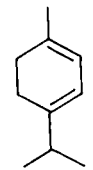
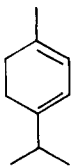
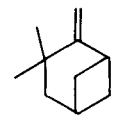
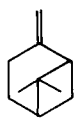
A medley of miscellaneous behavioral activities appearing to span virtually the known range of chemically based responses of ants is induced by one or more ant venoms. In addition to the trail pheromones previously discussed in detail, formicid venoms contain sex pheromones, alarm pheromones, aggregation pheromones, and queen pheromones, to list a few. Some ant venoms also contain allomones.

Sex pheromones are unlikely products of venom glands which, at least primitively, served primarily as producers of fluids for defense or prey capture. Nevertheless, Hölldobler (1971) identified the venom of the myrmicine *Xenomyrmex floridanus* as the first known source of an ant sex phermone. In this species, the large poison gland produces a pheromone that alone both attracts males and induces them to extrude their genitalia. Gasters without the venom reservoirs were unattractive. The venoms of alates of at least four species in the genus *Pogonomyrmex* are also the source of sex pheromones (Hölldobler, 1976b). Within the tribe Leptothoracini the venoms of *Harpagoxenus sublaevis* (Buschinger, 1972). *Formicoxenus nitidulus* (Buschinger, 1976), and a variety of species of *Leptothorax* (Buschinger, 1974; Möglich, 1979) contain sex pheromones. Species in these genera also respond to a venom-produced pheromone that attracts and also serves as the pheromone for tandem running. The pheromones among the 10 species investigated appeared to be specific at the subgenus level, but some cross-subgeneric specificity was also noted. The slave maker, *Harpagoxenus americanus*, also responded to the pheromones of the subgenus *Leptothorax*

Table XI

Monoterpene Hydrocarbons Identified in the Venoms of *Myrmicaria natalensis* and *M. eumenoides*[a]

Terpene	Relative quantity[b]		Terpene	Relative quantity[b]	
	M. natalensis	*M. eumenoides*		*M. natalensis*	*M. eumenoides*
 Limonene	Major component	36.4%	 β-Myrcene	Small, minor component	8.5%
 Sabinene	Second largest component	0.3%	 α-Phellandrene	Small, minor component	—

Terpene			Terpene		
 α-Pinene	Minor component	4.5%	 α-Terpinene	Small, minor component	—
 Terpinolene	Minor component	2.3%	 Camphene	Trace	—
 β-Pinene	Minor component	45.0%			

[a]Data from Brand *et al.* (1974); Howse *et al.* (1977).
[b]Quantities (top to bottom) are in order of decreasing quantity for *M. natalensis* and by percentage of total for *M. eumenoides* (— , not detected).

(Möglich, 1979). Because two elements of behavior, sexual attraction and attraction/tandem running, are both based on the same venom-releasing behavior within the tribe, this myrmicine tribe may represent a pivotal point in the evolution of ant pheromones. Tandem running is believed to be a primitive form of pheromonally based trail following behavior. Since the attractant behavior involved in the tandem running of *Leptothorax* is identical to the calling behavior ('*Locksterzeln*') of female *H. sublaevis*, its slave-making species, it is tempting to postulate that this behavior evolved in two directions, one into sex pheromones and the other into recruitment and trail pheromones (see Möglich, 1979, for further details).

Ant venoms are also known to elicit alarm behavior, presumably as a result of small volatile compounds. In addition to formic acid, the well-known alarm pheromone of many formicine ants, alarm pheromones are present in the venoms of some ponerine and myrmicine ants. The ponerine species *Leptogenys ocellifera* produces alarm and recruitment pheromones in its venom (Maschwitz and Mühlenberg, 1975). Crushed poison glands of another ponerine, *Pachycondyla laevigata*, induce an outrush of aggressively displaying workers, suggesting that an alarm pheromone is also present in this venom (Hölldobler and Traniello, 1980). Maschwitz (1964) reported the venom of *Myrmica* induces alarm and Cammaerts *et al.* (1978) identified a variety of volatile compounds including ethanal, acetone, and butanone, among others, from the venom and Dufour's glands of *Myrmica*. The latter authors describe these compounds as short-term attractants that increase exploratory action, activities characteristic of alarmed ants. Further studies are needed to elucidate specific details of this system.

Venoms of several species of *Pogonomyrmex*, when placed in a foraging area with the ants, elicit characteristic behaviors of alarm: rapid movement, head and antenna raising, and mandible opening. An odor easily detectable by the human nose is also noted from the venom (J. O. Schmidt, unpublished). *Myrmica* and *Pogonomyrmex* are sister genera that have similar alarm pheromones produced in the mandibular glands (McGurk *et al.*, 1966; Cammaerts *et al.*, 1982). They both also produce painful stings. These similarities of the two genera suggest the possibility of a common evolutionary origin for their pheromonal ant glandular systems.

Unique among the Hymenoptera venoms are the unusual terpenoidal venoms produced by ants of the genus *Myrmicaria*, opportunistic termite predators. Nine monoterpenes have been identified from *M. natalensis* (Grünanger *et al.*, 1960; Brand *et al.*, 1974) and six from *M. eumenoides* (Howse *et al.*, 1977) (Table XI). Although the six components in the venom of *M. eumenoides* were also the six major constituents in the venom of *M. natalensis*, their relative proportions were different (as exemplified by β-pinene being the major component in *M. eumenoides* and the fifth largest in *M.*

natalensis). These terpenes control recruitment, are repellent to other ants, are toxic to other ant species, and act as alarm pheromones (Howse *et al.*, 1977). Thus, they probably function well as part of the overall defense system of the species. The similarity of these terpene venoms and the defensive secretions of some termite species indicates that further investigations of their predatory behavior might bring forth further roles for the venom components.

Two aromatic nitrogen-containing compounds and three lactones have been isolated and identified from myrmicine ant venoms (Table XII). Skatole, 3-methylindole, is produced in the venom of soldiers of *Pheidole fallax*. Minor workers do not contain any of this caste-specific compound (Law *et al.*, 1965). The role of skatole was not clearly defined (it is not the trail pheromone, which is produced only by the minor workers) but, based on its repellent properties and the fact that soldiers are the main fighting force, the compound undoubtedly functions as a allomone. Anabaseine, a toxic alkaloid known from tobacco, is present in the venom of two *Aphenogaster* species. Like skatole, its role was not clearly defined, though it is not a trail pheromone and may be an attractant at low concentrations and a repellent at high concentrations (Wheeler *et al.*, 1981). Its role appears at this time to be primarily that of an allomone.

Queens of the fire ant *Solenopsis invicta* produce in their venom reservoirs the queen-recognition pheromones. These pheromones elicit orientation and attraction of workers to the source and promote clustering and the deposition of brood near the source (Vander Meer *et al.*, 1980; Glancey *et al.*, 1984). Rocca *et al.* (1983a,b) isolated three lactone queen-recognition pheromones from extracts of queens (Table XII). Although the lactones have not been unequivocally demonstrated to come from the poison reservoirs, the similarity of the pheromonal activities of the synthetic lactones and poison gland extracts suggests that the poison reservoir is the source of the phermones.

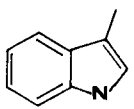
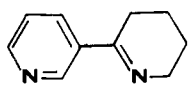
7. Active Polysaccharides in Ant Venoms

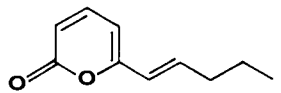
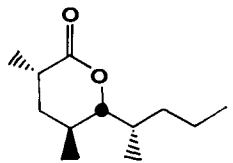
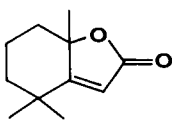
With one exception, polysaccharides represent a veritable unknown in ant venoms. This may result from lack of investigation of polysaccharides, or from a general overall uninterest in polysaccharides from a toxinological point of view. Nevertheless, one genus of ants, *Pseudomyrmex*, has been found to contain interesting and highly pharmacologically active polysaccharides in their venoms.

Crude venom (crushed sting apparatuses) of an unidentified South American species of *Pseudomyrmex* was found to be active against the human complement system. The venom preparation and fractions were found to cause decreases in the levels of the complement components C_4, C_2 and C_{3b}

Table XII

Small, Not Previously Listed, Biologically Active Molecules Isolated from Ant Venoms

Venom constituent	Ant species	Function	Reference
 Skatole	*Pheidole fallax*	Allomone	Law *et al.* (1965)
 Anabaseine	*Aphenogaster fulva, A. tennesseensis*	Allomone (?)	Wheeler *et al.* (1981)

 (*E*)-6-(1-Pentenyl)-2*H*-pyran-2-one	*Solenopsis invicta*	Component of queen recognition pheromone	Rocca *et al.* (1983a)
 Tetrahydro-3,5-dimethyl-6-(1-methylbutyl)-2*H*-pyran-2-one	*Solenopsis invicta*	Component of queen recognition pheromone	Rocca *et al.* (1983b)
 Dihydroactinidiolide	*Solenopsis invicta*	Component of queen recognition pheromone	Rocca *et al.* (1983a)

INA (inactivator of C_{3b}). Two fractions of molecular weights 175,000 and 32,500 were isolated and both contained the same two active components. One component was heat labile, hydrolyzed α-*N*-benzoyl-DL-arginine *p*-nitroanilide HCl (BAPNA), and was inhibited by soybean trypsin inhibitor and phenylmethylsulfonylfluoride, and digested purified C_2 and C_{3b} INA. This component was thus a trypsinlike enzyme (Schultz and Arnold, 1977, 1978). The other component contained a heterogeneous polyanionic polysaccharide (reacted to periodic acid Schiff reagent) that contained hexuronic acid but no sulfate or phosphate groups (not precipitated by Ba^{2+} salts). This fraction consisted of polysaccharides containing a large percentage of mannose plus *N*-acetylglucosamine, galactose, fucose, *N*-acetylgalactosamine, and glucose. A smaller polysaccharide with a molecular weight of 3000 could be obtained from the venom preparation, which also possessed glucosidase activity. This low molecular weight fraction, like the larger polysaccharides, could activate C_1, resulting in the loss of C_4 and C_2 (Schultz *et al.*, 1979).

Further hydrolysis of the polysaccharides by the glucosidase-containing venom preparation yielded polysaccharides of molecular weight 840 and 340. These oligosaccharides contained no lipid or hexuronic acid and both could activate C_1. Both oligosaccharides contained glucose, mannose, *N*-acetylglucosame, galactose, and *N*-acetylgalactosamine in ratios of 5:2:1:trace:trace. Hence, the smaller molecule is probably a subunit of the larger. Both are probably mixtures of compounds (Dieminger *et al.*, 1979).

The studies of *Pseudomyrmex* venom preparations indicate that this predominantly proteinaceous venom nevertheless contains highly active polysaccharides. The proportion of the polysaccharides in the venom is unknown, as well as the exact size and number of molecular species in the pure, unmodified venoms. Suffice it to say that the venom of *Pseudomyrmex* contains a storehouse of interesting chemicals including polysaccharides, which are active in a variety of systems.

B. Proteinaceous Venoms

Until now, only cursory mention has been made of protein-rich ant venoms. These venoms are probably found in a majority of ant species, including members of the following subfamilies: Ponerinae (Hermann and Blum, 1966), Myrmeciinae (de la Lande *et al.*, 1965; Cavill *et al.*, 1964), Pseudomyrmecinae (Blum and Callahan, 1963), Ecitoninae (Hermann and Blum, 1967b), and some of the Myrmicinae (Hermann and Blum, 1967a; Jentsch, 1969). Some of these studies have progressed little beyond determining that the venoms are proteinaceous. This is in large part an outcome of the fact that of all the proteinaceous arthropod and vertebrate venoms, those of the ants rank

among the most onerous to obtain in quantity and/or purity to study in detail. Nevertheless, based on the available information on the proteinaceous venoms of three ant genera, we can say that ants are exceedingly interesting toxinologically, and some species may well represent the pinnacle of venom development within the social insects. The genera studied in greatest detail are *Myrmecia*, a genus in the primitive subfamily Myrmeciinae, and *Myrmica* and *Pogonomyrmex*, members of the most derived subfamily of stinging ants.

1. Enzymes

Virtually all proteinaceous insect venoms that have been studied contain one or more enzymes. Ant venoms are no exception; indeed, they contain a staggering array of different enzymes, whose function and lack of correlation with taxonomy are often perplexing. All hymenopterous venoms that are used primarily for defense contain phospholipases and hyaluronidase. The one possible exception is the venom of *Myrmica ruginodis*, from which phospholipase is reportedly absent (Jentsch, 1969). However, based on personal experience with venoms of the related genus *Pogonomyrmex*, which has exceedingly high levels of phospholipase A (PLA) and phospholipase B (PLB), as well as otherwise inducing similar responses in stung humans, I believe further study will reveal phospholipases in *Myrmica* venom as well.

The distribution of phospholipase and hyaluronidase within the venoms of ants is shown in Table XIII. The activities are listed semiquantitatively where possible and as a 'p' for present where quantitative estimates are not practical. Ants generally possess low levels of PLA as compared to social wasps or the honeybee (J. O. Schmidt, M. S. Blum, and W. L. Overal, unpublished). One exception is the venom of *Pogonomyrmex*, which possesses very high levels of PLA (Schmidt and Blum, 1978a,b). Even the alkaloidal venom of the fire ant, *Solenopsis invicta*, contains PLA, though its quantitative level was not indicated (Baer *et al.*, 1979).

Unlike honeybee venom, most ant venoms contain at least a trace of PLB (Table XIII). In the case of the venom of *Solenopsis*, a question mark is enclosed behind the PLB listing. This is because a photograph in the report showed what clearly appeared identical to PLB as reported by Schmidt and Blum (1978b) for the venom of *Pogonomyrmex badius*. In that case strong evidence was presented that the two phospholipases were separate molecules (or on different parts of the same molecule) (Schmidt and Blum, 1978b).

Hyaluronidase, a ubiquitous enzyme in defensively used insect venoms, like PLA, is generally present in lower levels in the venoms of ants than social wasps and bees (J. O. Schmidt, M. S. Blum, and W. L. Overal, unpublished). Exceptions to this generalization are the venoms of *Dinoponera grandis* and *Pogonomyrmex badius*, which contain very high levels of the enzyme (Schmidt

Table XIII

Phospholipases and Hyaluronidase in Ant Venoms

Species	Relative activity[a,b]			Reference
	PLA	PLB	HYAL	
Myrmecia pyriformis	+	0	t	Lewis *et al.* (1968); Wanstall and de la Lande (1974)
Dinoponera grandis	t	t	++	J. O. Schmidt, M. S. Blum, and W. L. Overal (unpublished)
Odontomachus hematodus	t	t	+	J. O. Schmidt, M. S. Blum, and W. L. Overal (unpublished)
Paraponera clavata	+	t	t	J. O. Schmidt, M. S. Blum, and W. L. Overal (unpublished)
Ectatomma quadridens	+	t	+	J. O. Schmidt, M. S. Blum, and W. L. Overal (unpublished)
Ectatomma tuberculatum	+	t	+	J. O. Schmidt, M. S. Blum, and W. L. Overal (unpublished)
Platythyrea cribrinodis	++	t	+	J. O. Schmidt, M. S. Blum, and W. L. Overal (unpublished)
Pseudomyrmex mexicanus	++	++	+	J. O. Schmidt, M. S. Blum, and W. L. Overal (unpublished)
Eciton burchelli	+	t	+	J. O. Schmidt, M. S. Blum, and W. L. Overal (unpublished)
Solenopsis invicta	p	p(?)	p	Baer *et al.* (1979)
Myrmica ruginodis	0	0	p	Jentsch (1969)
Pogonomyrmex badius	+++	++	++	Schmidt and Blum (1978a,b)

[a]Relative activity levels: +++, very high; ++, high; +, low to intermediate; t, trace; p, present, but quantity not easily estimated; 0, not detected.

[b]Abbreviations: PLA, phospholipase A; PLB, phospholipase B; HYAL, hyaluronidase.

and Blum, 1978a,b; J. O. Schmidt, M. S. Blum, and W. L. Overal, unpublished). It is interesting that the activity levels of the phospholipases and hyaluronidase are not correlated in ant venoms. For example, the venom of *Dinopnera* contains the greatest activity of hyaluronidase of any tested ant species, yet its PLA level is very low.

The functions of these two apparently omnipresent enzymes in ant venoms is not entirely clear. Venom phospholipases in at least some species of stinging insects (e.g., the honeybee) (Habermann, 1971) are toxic. They cleave the fatty acids from phospholipids, releasing lysophopholipids that, in turn, act on membranes, such as those of erythrocytes and mast cells, to induce lysis (Robinson, 1961). The liberated free fatty acids can serve as precursors for the synthesis of prostaglandins (Kunze and Vogt, 1971) and leukotrienes (Samuelsson, 1983), pain-inducing and pharmacologically active substances. The function of hyaluronidase in venoms has long been ascribed to that of a spreading agent. That is, by hydrolyzing hyaluronic acid and chondrotin sulfate, macropolysaccharide polymers constituting the bulk of animal connective tissue, the enzyme opens passages through the host tissue matrix through which other venom components can diffuse. The identity of hyaluronidase as a spreading factor was first demonstrated by Chain and Duthie (1940) and was subsequently confirmed by Tu and Hendon (1983), who used purified hyaluronidase from the venom of the Mexican beaded lizard, *Heloderma horridum* to induce increased spreading of a hemorrhagic protein.

An assortment of enzymes other than phospholipases and hyaluronidase are also present in ant venoms. Acid phosphatase is present in all nine formicid species we have examined and appears to be a characteristic venom constituent within the family (Table XIV). This enzyme is either absent or present in very low relative quantities in social wasp and bee venoms (J. O. Schmidt, M. S. Blum, and W. L. Overal, unpublished). Extremely high levels were discovered in the venom of the white-headed soldiers (those with the ice-tong–like mandibles) taken from raiding columns of *Eciton burchelli*. High levels were also found in the venoms of *Ectatomma quadridens* and *Pogonomyrmex badius* (J. O. Schmidt, M. S. Blum, and W. L. Overal, unpublished).

The remainder of the venom enzymes listed in Table XIV exhibit rather sporadic occurrences within ant venoms. Alkaline phosphatase was detectable in only two ant venoms, and in those cases only in the most minute of trace quantities. Alkaline and acid phosphate activities appear not to be correlated in ant venoms: *Eciton* contains the highest known levels of acid phosphatase and no alkaline phosphatase, whereas *Paraponera* contains the lowest detected level of acid phosphatase and yet was one of the two venoms to possess alkaline phosphatase activity.

Phosphodiesterase, a common enzyme in snake venoms (Tu, 1977), was

Table XIV

Other Enzymes Detected in Ant Venoms[a]

Species	Relative activity[b,c]					
	AP	ALKP	PDE	LIP	EST	PROT[d]
Dinoponera grandis	+	0	0	t	0	0
Odontomachus hematodus	t	0	—	—	—	—
Paraponera clavata	t	t	t	0	0	0
Ectatomma quadridens	++	0	t	++	0	+
Ectatomma tuberculatum	++	0	t	+	0	t(?)
Platythyrea cribrinodis	++	0	0	+	0	0
Pseudomyrmex mexicanus	+	0	0	0	0	0
Eciton burchelli	+++	0	—	0	0	+++
Pogonomyrmex badius	++	t	0	++	++	0

[a]J. O. Schmidt, M. S. Blum, and W. L. Overal (unpublished).

[b]Relative activity levels: +++, very high; ++, high; +, low to intermediate; t, trace; 0, not detected; —, not analyzed.

[c]Abbreviations: AP, acid phosphatase; ALKP, alkaline phosphatase; PDE, phosphodiesterase; LIP, lipase; EST, esterase; PROT, nonspecific protease.

[d]Data from Schmidt (1984).

first discovered in insects in the venoms of ponerine ants in the genera *Paraponera* and *Ectatomma* (J. O. Schmidt, M. S. Blum, and W. L. Overal, unpublished). In these venoms the levels are very low, which suggests they do not play a major role.

The first animal venom reported to possess meaningful levels of lipase was that of an ant (Schmidt and Blum, 1978a). Subsequently, this enzyme was found to be present in a variety of ant and social wasp venoms (Schmidt, 1982; Table XIV). The venom of *Pogonomyrmex badius* possesses the highest lipase activity among ants followed by that of *Ectatomma quadridens*.

The enzyme esterase is distinguished from lipase by its specificity for cleaving ester linkages from short-chained fatty acids (two carbons in this case) rather than long-chained fatty acids (i.e., 18 carbons as in lipase). Esterase activity is rare among ant venoms and spotty among the venoms of social wasps and bees (J. O. Schmidt, M. S. Blum, and W. L. Overal, unpublished; Benton 1967). Only the venom of *Pogonomyrmex badius* contains the enzyme, and there it is present in high quantities.

The last enzyme analyzed was protease. This enzyme is present in high levels in the venom of soldier *Eciton burchelli* and in low levels in the venom of *Ectatomma quadridens*. A sister species, *Ectatomma tuberculatum*, appeared to possess the enzyme at the levels of the lower detection limit of the assay. Only one other species, *Polistes infuscatus*, a social wasp, possessed venom protease activity (Schmidt, 1984).

The roles in venoms of virtually all of these less common enzymes are unknown. Whatever their roles, this wealth of components undoubtedly plays some role in the biology of the ant species producing them. It should also provide fruitful areas for future biochemical and toxinological studies.

2. Other Proteins and Peptides

Whenever proteinaceous venoms are analyzed electrophoretically, a variety of constituents are visible with protein stains. Part of the mystery inherent in studies of venoms is the correlation of such protein bands with function. Unless the protein exhibits some enzymatic, physiological, or pharmacological activity that is measureable, its chemistry and biological properties are usually ignored. In this section, the chemistry of nonenzymatic proteins and peptides in ant venoms will be discussed.

Only one nonenzymatic venom protein has been isolated from an ant venom. This protein, given the trivial epithet of barbatolysin, is a potent hemolysin present in the venom of *Pogonomyrmex barbatus*. It is capable of actively disrupting the membranes of erythrocytes from a variety of mammalian species (Bernheimer *et al.*, 1980). The molecule is an extremely basic (isoelectric point at pH 10) peptide of molecular weight ~3000. The isolated peptide was found to contain ~34 amino acids, none of which contained sulfur. This molecule is a single chain of amino acids without cross-links (Bernheimer *et al.*, 1980). In earlier studies the hemolysin of *P. badius* was found not to complex with the polyanion heparin. In this regard its chemistry is strikingly different from that of melittin, the hemolysin in honeybee venom (Schmidt and Blum, 1978c).

Only scant chemical data are available on peptides from other ant venoms. Cavill *et al.* (1964) fractionated the venom of *Myrmecia gulosa* at pH 6.24 using paper electrophoresis. At this pH, two protein staining spots with kininlike activity by the guinea pig ileum and rat uterus assays were detected migrating slightly toward the cathode. They also detected a direct hemolytic protein, which migrated toward the anode.

Smooth muscle-stimulating activity, red cell-lysing activity, and histamine-releasing activities of another 'bull' ant, *Myrmecia pyriformis*, were chemically analyzed by Lewis and de la Lande (1967). They found that all three activities in the venom were slowly lost when it was boiled at pH 6.0, that trypsin and chymotrypsin destroyed most of their activity, and that all three activities were slowly dialysable through cellophane membranes. On ascending paper chromatograms run with water-butanol-acetic acid (5:4:1) and on low voltage paper electrophoresis at pH 6.24 all three activities exhibited similar movement. In a later study, starch gel electrophoresis at pH 4.5 was used

to separate the same venom into seven cathode-migrating fractions (Wanstall and de la Lande, 1974). The fifth most mobile of these fractions possessed the smooth muscle-stimulating activity. Using Sephadex G-50 gel filtration, the authors then determined that activities responsible for lysing erythrocytes, stimulating smooth muscles, and releasing histamine from mast cells all appeared in a fraction with a peak at molecular weight approximately 11,000. They thus concluded that the three activities probably resided in the same protein or peptide (Wanstall and de la Lande, 1974).

Neurotoxic activities are also present in a few ant venoms. At present, almost nothing is known of the chemical properties of any of these presumably peptidal components except that heparin does not complex with the neurotoxin in venom of *Pogonomyrmex badius*, which is responsible for the lethality to mice (Schmidt and Blum, 1978a,c). This, or a similar neurotoxin, was possibly localized in a fraction of venom from *P. barbatus* that had a molecular weight of 8000 and an isoelectric point of pH 8.0 (Bernheimer *et al.*, 1980).

3. *Small Biologically Active Compounds*

Social and solitary wasp and bee venoms frequently contain small biologically active compounds including histamine, 5-hydroxytryptamine, acetylcholine, norepinephrine, and dopamine (see Chapters 6 by Nakajima, 5 by Piek and Spanjer, and 7 by Shipolini and Banks, this volume). Although ant venoms have not been frequently analyzed for the presence of these constituents, in terms of active amine content, ants may be poor cousins in comparison to wasps and bees. Of all the above-mentioned compounds, only histamine has been detected in ant venoms. Both *Myrmecia gulosa* and *M. pyriformis* contain ~2% histamine, a fairly high level, in their venom (Cavill *et al.*, 1964; de la Lande *et al.*, 1965). The venoms of *Myrmica ruginodis* and *Pogonomyrmex badius* also contained histamine, though the amounts were not quantitated (Jentsch, 1969; Schmidt and Blum, 1978b). No 5-hydroxytryptamine was detectable in the venoms of *Odontomachus hematodus*, *Odontomachus* (= *Stenomyrmex*) *emarginatus* (Welsh and Batty, 1963), *Myrmecia pyriformis* (de la Lande *et al.*, 1965) or *Pogonomyrmex badius* (Schmidt and Blum, 1978b). Acetylcholine was also lacking in the venom of *M. pyriformis*.

VI. PHYSIOLOGICAL AND PHARMACOLOGICAL ACTIVITIES OF ANT VENOMS

Were it not for the pharmacological and physiological activities of ant venoms (or any other venoms), there would be little or no interest in them. Without such activities, venoms would be just another secretion. Ant venoms

are far from just another secretion, they exhibit a dazzling diversity of physiological and pharmacological activities. In terms of many absolutes, ant venoms take second place to those of no other insect group.

Many, but not all, of the experiments pertaining to the activities of ant venoms are rather basic compared to those performed on venoms of some of the parasitic wasps, social wasps, and especially, honeybees. This is partly a result of the difficulty of obtaining quantities of high-purity ant venom, partly because of the typically cryptobiotic and unobtrusive nature of stinging ants in Europe, temperate North America, and Japan, and partly a result of long histories of fine work done on bees, parasitic wasps, and social wasps mainly in Europe, but elsewhere as well.

In the following subsections, some of the pharmacological and physiological information on ant venoms will be summarized.

A. Paralytic and Lethal Activities of Ant Venoms against Arthropods

The differences in the physiologies of arthropods and vertebrates are so great that separate discussions of the activities of ant venoms toward each seem in order. Unlike the vertebrate situation, the distinctions between paralysis and death are often difficult to distinguish in arthropods. In vertebrates, paralysis often affects either the respiratory or circulatory system and a failure of either usually means rapid death. Arthropods, on the other hand, often appear dead when in fact they are simply paralyzed. Close scrutiny for feeble movements of mouthparts or tarsi often distinguishes the two, but similar types of movements may be also exhibited by dying insects. Thus, the only sure ways to test for paralysis in arthropods are either to wait to see if appendage flexibility decreases and/or decomposition occurs or to dissect the animal and look for internal signs of life or excitability within the nervous or muscular systems. No studies of the latter type have been reported for arthropods envenomated by ants. For these reasons, ant venom-induced paralysis and lethality to arthropods will be treated as a unit with distinctions made whenever possible.

A listing of ant species known to use their venoms to paralyze or kill arthropod prey was presented earlier (Table II). A quick glance at the table reveals that the majority of these belong to members of the subfamilies Ponerinae and Myrmeciinae. This agrees with a general consensus of myrmecologists that ant venoms evolved from a primitive use for prey capture to the more advanced use primarily for defense.

Cerpachys augustae, a representative of a ponerine tribe that often preys on termites or on ants such as *Pheidole*, was noted early in this century to induce sudden paralysis in stung prey (Wheeler, 1903). This paralyzing activity was studied in depth in a species in the *C. turneri* group. This species preys

exclusively on adults and larvae of *Pheidole*. Stung adults of *Pheidole* appeared to be almost immediately paralyzed and, interestingly, the larvae and pupae were also stung. Stung larvae and pupae were subsequently stored in the *Cerpachys* nest for up to two months (Hölldobler, 1982). Unstung larvae kept under identical conditions to the paralyzed larvae were dead within 2 weeks, whereas the paralyzed individuals still moved mouthparts when stimulated. In this group, the venom is clearly not lethal in activity, but resembles some of the solitary wasp venoms by inducing permanent and irreversible paralysis.

Leptogenys neutralis, a member of another ponerine tribe, also paralyzes its prey and sometimes stores excess paralyzed prey in a small chamber apart from the brood. Again as in the case with *Cerapachys*, paralyzed prey could sometimes move their antennae and legs when stimulated (Wheeler, 1933). In the case of *L. chinensis*, a termite predator, stung prey were immediately paralyzed and placed in a pile within the colony. These termites could also react to stimulation at least a day after being envenomated (Maschwitz *et al.*, 1979). Similar venom-induced paralysis by *Harpegnathus saltator* was reported; in this case a paralyzed cockroach could still respond after 2 weeks. No prey ever recovered from paralysis by either species (Maschwitz *et al.*, 1979).

Amblyopone pallipes, a representative of perhaps the most ancestral (primitive) tribe of ponerines, appears to paralyze its prey more slowly than the previous two genera. Paralysis is observed spreading from around the sting site and is usually followed by continued stinging until prey movement ceases. Prey are maintained undecomposed until consumption (Traniello, 1982).

The evidence for or against paralysis in most of the remaining taxa of ponerines and myrmeciines listed in Table II is weak; often all that is noted is that the sting is used for prey capture. In the case of the myrmicines, evidence is also weak. In this subfamily, however, the venoms probably are more lethal than paralytic: *Solenopsis* and *Wasmannia* readily use their stings against predators and competing ant species, but the frequency of venom use when foraging away from the colony is unclear. When used for defense, the venoms of both species appear to be lethal to other ants rather than paralytic (Sannasi and Blum, 1969; Bhatkar *et al.*, 1972; Howard *et al.*, 1982).

One myrmicine venom that is not particularly lethal or paralytic to arthropods is that of *Pogonomyrmex badius*. When injected into larval *Sarcophaga* and *Galleria*, respective LD_{50} values of 58 and 103 $\mu g/g$ were obtained (Schmidt and Blum, 1978c). At that level of lethality, the entire venom of one ant ($\sim 15 \mu g$) could kill only 50% of 0.15 to 0.26 g of prey, and immobilization was far from rapid. Greater venom doses increase the speed of knockdown, but not enough to make a crucial difference in most cases. Thus, the venoms of these predominantly nonpredatory ants appear

to have lost meaningful paralytic or lethal function toward arthropods and have taken on new roles (defense against vertebrates).

From the information available and presented above, a picture of ancestral use of paralyzing venom evolving toward use as a lethal defense and perhaps finally away from use against arthropods entirely appears to be occurring. This scenario is somewhat oversimplified in that only one aspect of ant venoms is considered. When parsimonious roles of venom such as defense against vertebrates, algogenicity (pain-inducing potential), and communication are considered, a picture of the great evolutionary plasticity of ant venoms emerges.

B. Lethal Toxicity and Paralytic Activities against Vertebrates

Death rather than paralysis is the most frequently observed cause of immobilization of vertebrates by ant venoms. The specific neurological or physiological causes of death resulting from ant or insect venoms are rarely known. In most cases, the animal ultimately dies of apneic convulsions following decreased respiratory function. In situations in which high doses of venom are administered, the animal often dies rather quickly, presumably due to neurotoxic activities of the venom. These observations are particularly noted when intravenous injections are made. Mice envenomed with equivalent to twice the LD_{50} quantity of *Pogonomyrmex* venom frequently die within 20 min of clonic convulsions and with exophthalmia (eyes protruding) (J. O. Schmidt, personal observations).

The lethality of hymenoptera venoms including those from ants are listed in Table XV. The listing includes representatives typical of each family or genus rather than all known species. The venoms of solitary wasps and bees are generally of low lethality. This is not surprising, as these species have no large colony structures to defend against large predators; they need only personally defend themselves (see earlier discussion on roles of venoms). Although few social bee venoms have been analyzed, their venoms appear to be reasonably lethal, suggestive of an important role in colony defense. The social wasps' venoms represent a real potpourri of activities that do not especially conform to our expectations of colony defensive needs. For example, *Dolichovespula* and especially *Polybia* live in huge colonies, yet have venoms of low lethality. *Polistes infuscatus* lives in smaller colonies, yet its venom is much more lethal.

Of all the insect venoms, those from two genera of ants are the most lethal. The venoms of *Ectatomma tuberculatum* and especially *Pogonomyrmex* are more lethal to mammals by a factor approaching 10 times that of the venoms of the next most lethal insects. The least lethal ant venoms in Table XV are those of *Dinoponera* and possibly *Myrmica*. *Dinoponera* does not sting

Table XV

Lethality of Venoms from Ants and other Hymenoptera to Mammals

Species (route of venom administration)[a]	LD_{50} (mice) (mg/kg)	Reference
Solitary wasps and bees		
Mutillidae		
Dasymutilla klugii (ip)	71	Schmidt *et al.* (1980)
Pompilidae		
Pepsis formosa pattoni (ip)	65	J. O. Schmidt (unpublished)
Anthophoridae		
Xylocopa virginica (ip)	22	Schmidt *et al.* (1980)
Social bees		
Apidae		
Bombus impatiens (ip)	7.2	Schmidt *et al.* (1980)
Apis mellifera (iv)	3.5	Neumann and Habermann (1954)
Social wasps		
Vespidae		
Apoica pallens (ip)	59	Schmidt *et al.* (1980)
Polybia sericea[b] (ip)	32	Schmidt *et al.* (1980)
Polistes infuscatus (ip)	6.6	Schmidt *et al.* (1980)
Ropalidia sp (ip)	13.7	J. O. Schmidt and P. J. Schmidt (unpublished)
Dolichovespula maculata (ip)	50	Schmidt and Blum (1979)
Vespa mandarinia (iv)	4.5	J. O. Schmidt and S. Yamane (unpublished)
Paravespula pensylvanica (iv)	6.4	J. O. Schmidt (unpublished)

Ants		
Formicidae		
Myrmeciinae		
Myrmecia nigriceps (ip)	7.3	J. O. Schmidt and P. J. Schmidt (unpublished)
Ponerinae		
Dinoponera grandis[c] (ip)	38	Schmidt *et al.* (1980)
Paraponera clavata (ip)	6.0	Schmidt *et al.* (1980)
Ectatomma tuberculatum (ip)	1.7	Schmidt *et al.* (1980)
Pseudomyrmecinae		
Pseudomyrmex mexicanus (ip)	8.0	J. O. Schmidt (unpublished)
Ecitoninae		
Eciton burchelli (ip)	10	Schmidt *et al.* (1980)
Myrmicinae		
Myrmica ruginodis (iv)	50–60	Jentsch (1969)
Pogonomyrmex (range 15 spp.) (ip)	0.10–0.62	Schmidt *et al.* (1980) J. O. Schmidt and P. J. Schmidt (unpublished)
(range 13 spp.) (iv)	0.12–1.1	Schmidt *et al.* (1980) J. O. Schmidt and P. J. Schmidt (unpublished)

[a]Abbreviations: ip, intraperitoneal; iv, intravenous.
[b]Previously called *P. chrysothorax* in Schmidt *et al.* (1980).
[c]Previously called *D. gigantea* in Schmidt *et al.* (1980).

humans (except perhaps if directly forced to sting) and is rather mild in disposition compared to the majority of large ponerines. *Myrmica* reportedly stings painfully (Jentsch, 1969). The estimate of its lethality was derived from a crude venom preparation, and hence, the value in Table XV may not reflect the true value for the venom of *Myrmica*.

The lethality of the venom of *Pogonomyrmex* is especially striking. The lethality appears to be characteristic of the genus rather than occurring in isolated species. By the intraperitoneal (ip) route of administration, the venoms of all 15 tested species were well below 1 mg/kg in lethality (Schmidt *et al.*, 1980 and unpublished data). Since the intravenous (iv) route of injection is frequently reported for other venoms, this route was also used for venom of *Pogonomyrmex*. The lethality by iv was similar to, or slightly less than, that for ip. The times till death were strikingly different: iv-envenomed mice frequently died quickly, often within 60 min (or else survived); ip-envenomed mice died slowly, with deaths usually starting at 6 hr postinjection and peaking between 12 and 24 hr or even longer.

For comparison to other ant venoms, the intravenous minimum lethal dose (LD_{50}) of formic acid, the main active constituent of the topically applied venoms of the subfamily Formicinae, is 145 mg/kg (Malorny, 1969). This value indicates that even if formicine ants could sting and inject venoms, the venoms would be many times less active than any other reported ant venom.

C. Algogenicity

Perhaps the most difficult of all venom activities to measure is pain and its quantitation. Science and medicine have by no means unraveled the mysteries of pain (Wall and Woolf, 1980) and presently no simple, accurate means to measure acute pain in animals have been developed (Melzak, 1976). Unfortunately, measurement of pain still appears to be entrenched in the anecdotal stage and is best evaluated by time-consuming, complicated, and expensive surveys and questionnaires (Melzak, 1975). Nevertheless, pain is of great value in defense and its significance must not be overlooked. For these reasons, I have adopted a subjective and simple means of providing estimations of venom painfulness—a relative rating scale of pain intensity (Table XVI). Although this method has obvious drawbacks, not the least of which is low sample size and high variance between individual subjects, it does provide at least a modicum of useful information *vis-à-vis* this aspect of venom activity. For the scale in Table XVI, a value of 2 is given to the pain of a honeybee sting, the insect whose sting is probably the most familiar to the general public. Pain levels from particular stings do, of course, vary and depend on such features as where the sting occurred and how much

venom was injected; thus honeybee stings can range from a relative pain of 1+ (e.g., to the back of the hand followed by rapid stinger removal) to 3 (e.g., nose, eyes, lips, palm of hand). The values in Table XVI represent my personal impressions plus those of collaborators and people interviewed in the field. Duration times are also from personal experiences and wide departures may be experienced or reported by other individuals.

The sting pain resulting from envenomation by solitary wasps and bees is almost invariably of short duration. Even the exceedingly painful stings by tarantula hawks (*Pepsis*) last only a few minutes before the pain is entirely gone. Mutillid wasp stings are usually similar, though some people experience longer-lasting burning and/or swelling. Stings from ants, social bees, and wasps typically induce intense immediate pain, which usually lasts up to 10 min followed by a longer-lasting, low-level burning sensation. In most cases, the main differences among social species are the levels of the intense immediate pain, though major swelling may result and cause protracted inconvenience.

The venoms of only two species stand in contrast to those of the rest of the social insects. These are both ant venoms: *Paraponera clavata* and *Pogonomyrmex*. The former has such a reputation among the inhabitants in the Neotropics that it has acquired numerous local names including *tucandeira*, *cumanagata*, *munuri*, *conga*, *chacha*, *viente cuatro hora hormiga*, and *hormiga bala*. It was also used in some local rituals to test for manhood. The ants may be placed inside woven fiber cylinders into which young marriage-seeking men must place their hands and demonstrate ability to withstand pain (Lange, 1914). Alternatively, a woven mat containing these ants (and possibly other species) is applied to the young man's thorax, gluteus maximus, thigh, etc. (Weber, 1937). That *Paraponera* is familiar enough to be given common names and important enough to be used in rites clearly demonstrates the algogenic nature of its venom. I can attest from personal experience that *P. clavata* is by far the most painfully stinging of all hymenopterans (if any other species vies for that title, it obviously is very rare and has not been reported in the literature).

Stings by *Paraponera* induce immediate excruciating and totally debilitating pain. This pain generally persists unrelentingly with waves of intense pain for at least several hours, and often as long as 24 or more hr (Lange, 1914; Rice, 1914; Bequaert, 1926; Weber, 1937, 1939; Allard, 1951; Hermann and Blum, 1966; author's personal experience). Sometimes accompanying the pain are numbness, vomiting, inflammation, and uncontrollable trembling (Rice, 1914; Bequaert, 1926; Weber, 1937; Allard, 1951; Hermann and Blum, 1966; author's personal experience). The note by Allard (1951) needs further comment. The symptoms he described were completely congruent with those of *P. clavata*; yet he ascribed them to *Dinoponera grandis* (=*gigantea*), a

Table XVI

Relative Algogenicity to Humans of Envenomations by Various Hymenopterans

Species	Relative immediate sting pain[a]	Duration of pain
Solitary wasps and bees		
Scoliidae		
various spp.	0–1	Short
Pompilidae:		
Pepsis formosa pattoni	4	2–5 min
other spp.	2–3	Short
Mutillidae		
Dasymutilla lepeletierii	2–3	Short
Dasymutilla klugii	3	Short (usually)
Small species	1–2	Short (usually)
Sphecidae		
various spp. (excluding spider predators)	0–1	Short
Anthophoridae		
Diadasia r. rinconis	1–2	Short
Centris pallida	1–2	Short
Xylocopa virginica	2	Short
Small solitary bees in general	0–2	Short
Social bees		
Apidae		
Apis mellifera	2	4–10 min[b]
Bombus sonorus	2	2–5 min[b]
Social wasps		
Vespidae		
Polistinae		
Polistes fuscatus	2	2–5 min[b]
P. arizonensis	2–3	4–10 min[b]
P. infuscatus	3	5–10 min[b]
Polybia rejecta	2	2–4 min[b]
P. sericea[c]	2	2–5 min[b]
Apoica pallens	2	2–5 min[b]
Ropalidia sp.	1–2	1–3 min[b]
Vespinae		
Paravespula pensylvanica	2	4–10 min[b]
Vespula squamosa	2	4–10 min[b]
Dolichovespula maculata	2	2–5 min[b]
Vespa mandarinia	2	4–10 min[b]
Ants		
Formicidae		
Myrmeciinae		
Myrmecia nigriceps	2	2–5 min[b]
M. pyriformis	2, 2–3	—[d]

(continued)

Table XVI (*continued*)

Relative Algogenicity to Humans of Envenomations by Various Hymenopterans

Species	Relative immediate sting pain[a]	Duration of pain
Ponerinae		
Dinoponera grandis	1–2	2–10 min
Odontomachus hematodus	2	4–10 min
Pachycondyla (=*Neoponera*) *apicalis*	2	4–10 min
Ectatomma quadridens	2	—
Paraponera clavata	4	Intense 3–5 hr, less to 24+ hr
Pseudomyrmecinae		
Pseudomyrmex mexicanus	1–2	2–5 min
Ecitoninae		
Eciton burchelli	1–2	2–5 min
Myrmicinae		
Solenopsis invicta	1–2	2–5 min, prolonged local reaction
Myrmica hamulata	1–2	1–4 hr
Pogonomyrmex spp.	3	Intense 1–4 hr, less to 12 hr

[a]Relative pain scale: 0, cannot penetrate skin; 4, maximum.
[b]Frequently residual pain of longer duration.
[c]Previously named *P. chrysothorax* in Schmidt *et al.* (1980).
[d]Based on de la Lande *et al.* (1965) and Lewis and de la Lande (1967).

species reluctant to sting and, in any case, only moderately painful when it does sting (Schmidt *et al.*, 1980). The species was reportedly identified by M. R. Smith; however, on inquiry to Smith's museum (U.S. National Museum of Natural History), I discovered that Allard had sent many specimens, including *Paraponera*, for identification, with none labeled as those causing the reported reactions. Almost certainly the wrong species name had been used in the paper (D. R. Smith, personal communication).

Envenomations by species in the genus *Pogonomyrmex* are different in nature from those of *Paraponera* and also are rather unusual compared to stings from other Hymenoptera. From my own experience, unless I actually observe the ant stinging, I often do not detect the envenomation for an estimated 5–30 sec. Thereafter, however, the pain becomes exceptionally intense and piercing. This pain then maintains its high level for up to several hours and has been described by collaborators as like 'ripping muscles or tendons' or 'turning a screw' in the flesh around the sting site. The venom also frequently induces pain in the axial lymph nodes nearest the sting site and causes a difficult to characterize, decidedly unpleasant, but not sharp

or intense, sensation that can perhaps best be described in emotional terms such as 'chilling', causing 'muscle soreness', or 'being constantly present and exceedingly psychologically unaesthetic'.

Literature references to the stings of various *Pogonomyrmex* species are sometimes vivid and colorful. One of the earliest reports was that of McCook (1879) who lucidly described the sharp pain, the nervous, chilling sensations that swept upward from the sting site, and the heavy pain that lasted at least 3 hr after a sting from *P. barbatus*. Later reports including those by Wheeler (1910), Weber (1959), Wheeler and Wheeler (1963), Williams and Williams (1964), Hermann and Blum (1967a), and Cole (1968) have given generally similar reports of sting reactions.

D. Membrane Activity

Ant venoms frequently contain direct membrane-disrupting constituents. The most common assays for membrane-disrupting activity are based on the ability to lyse either erythrocytes, thereby releasing hemoglobin, which is spectrophotometrically measured, or mast cells, followed by measurement of their released histamine. The hemolytic assay is simpler and has been the more frequently used method. The actual known chemistry of the proteinaceous hemolysins from ant venoms was presented earlier.

Ant species known to possess directly hemolytic venoms and the relative activities of those venoms are presented in Table XVII. Eleven genera of ants have been reported to contain hemolysins in their venoms. Of these, seven are ponerines, two are myrmicines, and one each is a myrmeciine, an ecitonine, and a pseudomyrmecine. In other words, hemolytic activity appears to be distributed throughout the major groups of stinging ants.

The two most hemolytic of the studied ant venoms are those of the congenerics *Pogonomyrmex barbatus* and *P. badius*. Though similar, these venoms exhibited slight differences in their activities toward washed erythrocytes from 11 species of mammals. When assayed with the venom of *P. barbatus*, dog and mouse cells were the most sensitive (900 and 850 hemolytic units/mg venom) and sheep and goat cells were the least sensitive (210 and 185 units/mg venom). The species differences appeared to be due to the preferential affinity of the lysins for erythrocytes in which phosphatidylcholine, rather than sphingomyelin, is the major external phospholipid (Bernheimer *et al.*, 1980).

The hemolytic activities of most ant venoms, including those of *Pogonomyrmex*, are lower than those of social wasps (Schmidt *et al.*, 1984a). The structures of the hemolysins in wasp and bee venoms (Bernheimer *et al.*, 1982; Habermann and Jentsch, 1967) are also different from those of

Table XVII
Hemolytic and Neurotoxic Activities in Ant Venoms

Species	Relative activity[a]		Reference
	Hemolytic activity	Neurotoxic activity	
Myrmecia gulosa	p	—	Cavill *et al.* (1964)
Myrmecia pyriformis	p	—	Lewis and de la Lande (1967)
Dinoponera grandis	+	0[b]	Schmidt *et al.* (1984a)
Odontomachus hematodus	+	0[b]	Schmidt *et al.* (1984a)
Paraponera clavata	t	+[b]	Schmidt *et al.* (1984a)
Ectatomma quadridens	+	0[b]	Schmidt *et al.* (1984a)
Ectatomma tuberculatum	+	0[b]	Schmidt *et al.* (1984a)
Platythyrea cribrinodis	++	0[b]	Schmidt *et al.* (1984a)
Pachycondyla (= *Neoponera*) *apicalis*	+	0[b]	Schmidt *et al.* (1984a)
Pseudomyrmex mexicanus	++	0[b]	Schmidt *et al.* (1984a)
Eciton burchelli	t	0[b]	Schmidt *et al.* (1984a)
Solenopsis	p	+	Adrouny *et al.* (1959); Yeh *et al.* (1975)
Myrmica ruginodis	0[b]	++	Jentsch (1969)
Pogonomyrmex badius	++	++	Schmidt *et al.* (1984a)
Pogonomyrmex barbatus	+++	++	Bernheimer *et al.* (1980)

[a]Relative activity levels: +++, very high; ++, high; +, low to intermediate; t, trace; p, present, but quantity not easily estimated; 0, no evidence for activity; —, not reported.
[b]See text.

at least *P. barbatus* (Bernheimer *et al.*, 1980). One last difference between the hemolysins in *Pogonomyrmex* venom and those in wasp and bee venoms is the ability of heparin to inhibit the hemolytic activities of the latter venoms (Joshua and Ishay, 1973; Habermann, 1968), but not of the venom of *Pogonomyrmex* (Schmidt and Blum, 1978c). These differences indicate that the hemolytic activities of the venoms of *Pogonomyrmex*, and probably ants in general, had separate evolutionary origins and structures from those of bees and wasps but that, as a result of selection pressure, the activities of the three groups converged.

It would be most informative to compare in detail the hemolytic activities of various venoms within the Formicidae. The venom of *Myrmica*, the genus most closely related to *Pogonomyrmex*, would be of particular interest. Although a venom from one *Myrmica* species was reported to lack hemolytic activity, a reinvestigation would be worthwhile. Analyses of venoms from other subfamilies might also help elucidate the question of mono- versus polyphyletic origin of venom hemolysins within the ants.

The venom hemolysins of at least one genus, *Solenopsis*, definitely had

separate origins from those of the rest of the ants. Instead of proteinaceous hemolysins, the venom of the fire ant *S.* (presumably) *invicta* contains a crystalline, heat-stable hemolysin that is soluble in organic solvents (Adrouny *et al.*, 1959). The venom of *S. xyloni* also contains similar hemolytic activity (Blum *et al.*, 1961). The mechanisms by which these (piperidine) fire ant hemolysins act has not been elucidated, but they probably operate in a manner similar to the actions of fire ant venom, or venom piperidine, in lytically releasing histamine from mast cells. In the investigations involving mast cells, whole venoms from *S. geminata* and *S. invicta* or the pure piperidine *cis*-C_{11} caused histamine release from rat mast cells. Before releasing histamine, the cells displayed substantial swelling, even in metabolically inactivated cells (heated to 50°C for 15 min). These findings indicate that histamine release is caused by a permeability-perturbing action on the cell membrane that is nonspecific and is probably a function of the amphipathic character of the venom alkaloids (Lind, 1982). This same mechanism probably causes the hemolytic activity of fire ant venom alkaloids on erythrocyte membranes.

E. Neurotoxicity

Neurotoxic activity in the venoms of several species of ants can be inferred, but actual physiological demonstration or neurotoxicity is recorded in only one case. Table XVII provides a listing of the presence of neurotoxic activities (excluding paralysis of prey) in various ant venoms. Following an envenomation, symptoms including piloerection (*Pogonomyrmex*, *Myrmica*), sweating near the sting site (*Pogonomyrmex*, *Myrmica*), and uncontrollable trembling that was not caused by pain alone (*Paraponera*) were considered as evidence of neurotoxic activity. Local sweating and piloerection imply that the venom acts on the peripheral nervous system, and trembling suggests action on the deeper nervous or neuromuscular systems. Lack of any of these, or other symptoms indicative of specific action on nerves or muscles, was considered evidence for lack of neurotoxicity among the venoms listed in Table XVII. In addition to the species listed in the table, I have been stung by numerous other species without observing any, or at least any profound, neurotoxic activity.

In addition to the criteria mentioned above, the venoms of ants in the genus *Pogonomyrmex* exhibit potent neurotoxicity when injected into mice. The activity takes two forms: occasional rapid death resulting from tetanic convulsions (Schmidt and Blum, 1978c), and the extreme iv and ip lethality of the venoms (LD_{50} as low as 100 to 250 μg/kg). Such extreme lethality indicates the presence of specific activity directed toward excitable nervous or muscular tissue rather than activity of a nonspecific and destructive nature. The finding that the venom of *P. badius* is 100–200 times more lethal to mice

than to insects (Schmidt and Blum, 1978c) further reinforces the conclusion that lethality in mice is not a result of nonspecific venom action.

The only ant venoms for which detailed neurotoxic activity studies have been reported are those of fire ants. Koch and Desaiah (1975) and Koch *et al.* (1977) showed that both *Solenopsis richteri* venom (where *trans*-C_{15} and *trans*-C_{13} piperidines predominate) and synthetic *cis*-C_{15} and *cis*-$C_{15:1}$ piperidines (found in the venom of *S. invicta*) will inhibit (Na^+, K^+)-ATPase, oligomycin-sensitive Mg^{2+}-ATPase, and oligomycin-insensitive Mg^{2+}-ATPase. The length of the hydrocarbon side chain on the 6-position of the piperidine ring has an important role in the ATPase-inhibitory activity of these compounds (Koch *et al.*, 1977; Walsh and Koch, 1978). The results in this study suggest that fire ant venom has an uncoupling effect on oxidative phosphorylation. Cheng *et al.* (1977) examined this possibility in more detail using *cis*-C_{15}, a piperidine found in *S. invicta* venom. This compound, applied at 5 m*M* concentrations, uncouples cockroach mitochondrial oxidative phosphorylation in a manner similar to that of dicofol, a DDT-related acaricide. The toxicity of fire ant venom to other insects may therefore depend, at least in part, on the ATPase inhibitory and oxidative phosphorylation uncoupling activities of the piperidine components.

Further toxic effects of fire ant piperidines were demonstrated by Yeh (1973) and Yeh *et al.* (1975) in studies of the effects of these compounds on neuromuscular transmission. In the initial study, Yeh (1973) found that both the *cis*- and *trans*-C_{11}, -C_{13}, and -C_{15} piperidines would block neuromuscular transmission in sartorius nerve–muscle preparations of frogs. At a m*M* to 20 m*M* concentrations *cis*- and *trans*-C_{11} decreased the amplitudes of spontaneous miniature end plate potentials, nerve-evoked end plate potentials, and potentials induced by iontophoretic applications of acetylcholine. At 50 to 100 m*M* levels, the C_{11} compounds depolarized the end plate and non–end-plate regions of the muscle. Therefore, the effect of the piperidines on neuromuscular junction is postsynaptic. The C_{13} and C_{15} compounds were less effective and their lower activities were attributed to poor solubility (Yeh *et al.*, 1975). The same authors also characterized the postsynaptic blocking effect of *cis*- and *trans*-C_{11} piperidine derivatives. They demonstrated that there is little difference in the effects of the cis and trans isomers on activity and that the *trans*-C_{11} compound does not bind to the acetylcholine receptor site where α-bungarotoxin, *d*-tubocurarine, decamethonium, and carbamylcholine have their affinities. Thus, in the vertebrate system, the postsynaptic effects of piperidine compounds apparently follow the initial binding of acetylcholine to its receptor, possibly through an action on the coupling mechanism between the activated acetylcholine receptor and the increase in membrane ionic conductance. Krzanowski *et al.* (1982) treated canine tracheal smooth muscle preparation with 0.05 μg/ml of venom of

Solenopsis invicta and discovered that it blocked the ability of the toxin from *Ptchodiscus brevis* to induce contractions. However, veratridine and an unnamed scorpion toxin still induced contractions. Tetrodotoxin ($10^{-7}M$) blocked all contractions and acetylcholine responsiveness remained unblocked by the venom. The authors concluded that the venom of *S. invicta* blocks the *P. brevis* receptors of the postganglionic parasympathetic nerve axonal sodium channel and that these receptors are different from those for veratridine and the scorpion toxin.

In an insect system David *et al.* (1984) discovered that *cis*- and *trans*-C_{11} or C_{13} were not lethal to cockroaches, *Periplaneta americana*, when topically applied or when 200 μg per insect are injected. At concentrations of 1.3 to $1.6 \times 10^{-5}M$ the C_{11} piperidines and *trans*-C_{13} irreversibly block the response of fast coxal motoneurones to application of acetylcholine. The inhibition of acetylcholine-induced currents was independent of membrane potentials (in the range -120 to -60 mV), implying that the alkaloids do not interact with the membrane open receptor–ion channel complexes. Apparently in the cockroach the piperidines act on the closed acetylcholine receptor–ion channel complex, but at a site separate from that at which α-bungarotoxin binds.

F. Other Activities

Fire ant venoms display several venom activities not previously described. Whole venom of *S. richteri* successfully inhibited the growth of *Micrococcus pyrogenes*, *Streptococcus pyrogenes*, *Escherichia coli*, and *Lactobacillus casei*, plus a variety of molds (Blum *et al.*, 1958). *Trans*-C_{11}, -C_{13} and -C_{15} venom piperidines were found to have minimal bactericidal concentrations (MBC) of 8, 2, and 4 μg/ml respectively against *Staphylococcus aureus* and were also active against seven other species of gram positive bacteria in three genera (Jouvenaz *et al.*, 1972). Gram negative bacteria were much more resistant and *trans*-$C_{15:1}$ was inactive against any tested bacterial species. This antimicrobial activity is probably the reason for the sterility of the pustules found in human skin following envenomation by fire ants (Caro *et al.*, 1957). The ants also disperse small quantities (~ 1 ng) of venom to the surface of their brood, presumably to act as an antibiotic (M. S. Obin and R. K. Vander Meer, personal communication).

Fire ant venom causes the darkening of house fly larval hemolymph and cuticle. This blackening is not a consequence of death, because copper chelators such as ascorbic acid and phenylthiourea block the discoloration but do not reduce the lethality of the venom (Sannasi and Blum, 1969). The coloration appears to be the result of accumulations of melanins resulting from melanosis. Because small quantities of topically applied venom cannot

induce local coloration, the authors feel that the coloration results from formation of a definite pathological condition by a threshold or greater quantity of venom rather than by a direct action of the venom (Sannasi and Blum, 1969).

A final pharmacological activity unique among insect venoms is the ability of fire ant venoms to produce local necrosis in tissue. The necrosis is aseptic, indicative of venom rather than bacterial causes (Caro *et al.*, 1957). Usually within 24 hr of a sting by *Solenopsis invicta*, a pustule that contains polymorphonuclear leukocytes and lymphocytes forms at the sting site. Below the pustule is a layer of necroses, beneath which lie infiltrates of polymorphonuclear leukocytes and lymphocytes plus cells with small pyknotic nuclei. Later, eosinophilic leukocytes and plasma cells are found (Caro *et al.*, 1957). No immunoglobulin, G, A, M, E, or complement C3 were observed in the pustule fluid, indicating that immunological reactions were not involved in its formation. Quantitation of the cellular count within the fluid yielded values of 94% neutrophils and 6% lymphocytes in normal patients (deShazo *et al.*, 1984).

VII. POTENTIAL PHARMACOLOGICAL AND PHYSIOLOGICAL USES OF VENOMS

The most dramatic demonstration of the use of an ant venom to solve a problem was the use of the venom of *Pseudomyrmex* to alleviate rheumatoid arthritis. The case involved a woman with mixed cryoglobulinemia resulting in severe pain in her joints and who failed to improve with corticosteroids and chlorombucil and required frequent plasmaphoresis. Treatment with the venom of *Pseudomyrmex* caused improved in her condition and removal from the plasmaphoresis. The venom's anticomplement activity appeared to be the cause of the decrease in cryoglobulin levels and the return to closer to normal levels of complement factors C3 and C4. The authors suggest that this venom treatment may be warranted in patients suffering from cryoglobulinemia (Schultz *et al.*, 1978). Brown and Sisk (in Schultz and Arnold, 1978) also reported improvements for at least a year in another patient. Whether such treatments have continued to work or will prove efficacious in the future is not known. At the very least, such studies may pave the way via elucidating mechanisms of action for the development of better treatment using other materials.

Before the studies by Schultz and co-workers, ant and other insect venom use for treatment of arthritis was common in human societies (Weber, 1937). Such usage, though it does not confirm efficacy of venoms, does indicate widespread interest in such use of venoms. This perhaps could serve as a

starting point for investigating the pharmacologically active substances in ant venoms to see if they do, indeed, have any medical value.

A second medical use of ant venoms is the treatment of patients hypersensitive to ant venoms. At first the use of a venom to treat a problem caused by the same venom seems paradoxical, but it really is not. The problem of hypersensitivity would occur regardless; therefore, use of the venom to reduce the problem is beneficial. Specifically, in the United States some individuals suffer from hypersensitivity to the stings of fire ants or harvester ants (Triplett, 1973; Rhoades *et al.*, 1977; Pinnas *et al.*, 1977; Schmidt *et al.*, 1984b) and some of those sensitive to fire ants appear to have been successfully treated with whole body extracts (which contain some venom) of fire ants (Rhoades *et al.*, 1977). We are currently treating one patient for hypersensitivity to *Pogonomyrmex californicus* venom with pure venom and anticipate treating others in the future (J. L. Pinnas and J. O. Schmidt, unpublished). As more ant venoms, and especially fire ant venoms of greater purity, become available, this use can be expected to expand both in terms of frequency and geographical areas over which it is used.

In addition to the present limited direct use of ant venoms, there are numerous potential uses of the venoms or their constituents. The studies by Yeh *et al.* (1975) and Krzanowski *et al.* (1982) demonstrate the possible use of fire ant piperidine alkaloids as probes of the vertebrate neuromuscular system. The piperdines may also serve as agents for investigating enzyme systems involved in ion transport (Koch *et al.*, 1977; Walsh and Koch, 1978) or in energy generation (Cheng *et al.*, 1977). The complement activities of the venom of *Pseudomyrmex* continue to have potential for unlocking and controlling that complex system. The hemolysin in *Pogonomyrmex* venom has the potential of adding one more agent to the arsenal developed for investigating membranes, their structures, and their activities (melittin is another hymenoptera venom peptide that has already been used in such studies). The neurotoxin(s) in the venom of *Pogonomyrmex*, when isolated, also has potential as a new investigative tool for probing the mammalian nervous system and contrasting it with that of insects. And, finally, ant venoms might well serve as excellent sources of specialized enzymes such as lipase (in *Pogonomyrmex*), phospholipase A_2 (in many venoms), or hyaluronidase (in many venoms).

VIII. A CAVEAT: ANT VENOMS AND PEOPLE

Ant venoms are not only materials for scientific discovery and sources for expansion of our knowledge and understanding in a multitude of fields within the life sciences, but they also impact directly on human health and well being.

Aside from the painful nuisance of being stung, some ant venoms are allergenic and cause hypersensitive reactions, including some that are life-threatening. Venom allergy is essentially the immunologically-mediated hypersensitive reaction of the body to venom allergens, culminating in systemic reactions such as anaphylactic shock, respiratory distress, urticarial rash, angioedemal swellings, gastrointestinal distress, or neurological disorders. Most of these reactions occur after one or more initial 'sensitizing' stings and are immunologically mediated by sensitized immunoglobulin E (IgE), but there may be some exceptions that are not IgE mediated. Further details concerning the immunological basis of venom allergy (Fudenberg *et al.*, 1980), as well as the overall medical situation related to stings by hymenopterans (see Chapter 10) is beyond the scope of this report. The relationships between ant venoms and allergy will, however, be discussed in brief here, because ant venom-induced allergy is a specialized subdiscipline that receives little attention within the greater venom allergy field.

Ants have traditionally been overlooked as important medical problems because they are small, do not fly (except the generally harmless alates), and are difficult for nonentomologists and most entomologists to identify. Medical education and knowledge as a whole also has been lacking in understanding the ant problem (for example, many physicians not only still believe that all ant venoms are formic acid, but that venomous Hymenoptera in general contain formic acid). Nevertheless, ant stings are a serious problem in some countries, such as the United States. Clemmer and Serfling (1975) reported that 29% of 777 individuals surveyed in the New Orleans, Louisiana, United States area were stung by *Solenopsis invicta* within the previous year. In a rural county in the U.S. state of Georgia, 31% of 310 people surveyed were stung by the same species (Adams and Lofgren, 1981). A third study of 113 respondents indicated that between 35 and 58% of the inhabitants in a residential section of New Orleans had been stung during the previous year (deShazo *et al.*, 1984). These reports all indicate substantial envenomations of humans living in areas inhabited by fire ants. The areas inhabited by *S. invicta* contained 23 million human inhabitants according to a 1977 report (Schmid, 1977) and today probably contain in excess of 30 million inhabitants. If 30% of these are stung in a year, that indicates 9 million people per year are envenomated; even if the estimate is fourfold high, 2 million people are stung per year by fire ants. At a conservative estimate of 1 to 4.4% of stings being severe or requiring medical treatment (Adams and Lofgren, 1981; Clemmer and Serfling, 1975), 20,000–80,000 people require treatment per year. The actual rate is probably closer to the latter figure.

Harvester ants are the only other group of ants in North America whose stings sometimes require medical treatment. Although the stings of

Pogonomyrmex are much more painful and toxic than those of fire ants, the frequency of stings to humans is much lower. This probably is a result of three factors: the ants are generally less aggressive, they are large and conspicuous, usually with large, conspicuous, easily avoided mounds, and their stings hurt so much that most people take special precautions not to get stung repeatedly. Nevertheless, Micks (1960) reported 75 incidents of medically important ant stings in Texas, 27 of which were from harvester ants, two from fire ants, and 46 unknown (many presumably harvester ants). Other reports include a case of *P. barbatus* venom allergy (Lockey, 1974) and two examples of eight cases each of allergy to harvester ant venom (Pinnas *et al.*, 1977; Schmidt *et al.*, 1984b). No ant species outside the genera *Solenopsis* and *Pogonomyrmex* are confirmed to have caused allergic problems, though this may be partly an identification problem and partly because the population of other stinging ant species is low in areas of the world where such reports are likely.

Symptoms of fire ant venom allergy are frequently less severe than those of honeybee or vespid wasp venom allergy (Clemmer and Serfling, 1975; Stablein and Lockey, 1981; deShazo *et al.*, 1984). The most frequently reported symptoms are urticaria, 51–84%, and angioedema, 67–81% (Triplett, 1973; Rhoades *et al.*, 1977; Adams and Lofgren, 1982). Other symptoms are not systematically characterized within the various reports. In general, respiratory problems (difficulty in breathing, wheezing, etc.) occur in one-third to one-half the patients, cardiovascular involvement (collapse, dizziness, etc.) in about one-fifth, and gastrointestinal and neurological problems each in ~10% of patients (Triplett, 1973; Rhoades *et al.*, 1977). Actual death from ant venom allergy is exceedingly rare. In the United States, at most, there are 31 cases of reported deaths from ant stings (Bowen, 1951; Parrish, 1963; Brown, 1973; Lofgren *et al.*, 1975; Triplett in Rhoades *et al.*, 1977; Stablein and Lockey, 1981; and Hensel *et al.*, 1983). Of these, only seven are documented to any degree (Bowen, 1951; Parrish, 1963; Brown, 1973; Hensel *et al.*, 1983), and for five of them, the envenomating species is not reported (Bowen, 1951; Parrish, 1963). The general feeling is that most, or all, of these deaths are the result of stings by fire ants, with the exception of the case reported by Bowen (1951), in which *Pogonomyrmex* was almost certainly responsible.

In addition to allergy, medical problems related to ant venoms seriously impact humans economically. Death due purely to venom lethality has not been reported and is highly unlikely. For example, an inebriated male who became drowsy selected a fire ant mound as his pillow and received about 5000 stings. Excluding his 'hangover', he was fine the next morning (Smith

and Smith, 1971). In another case, a 1.5-year-old infant was stung 220 times by *Solenopsis invicta* without problems (Olive, 1960). The nonallergic medical problems usually relate to the 86 to 88% incidence of pustule formation following stings by *S. invicta* (Rhoades *et al.*, 1977; Adams and Lofgren, 1982). The overall cost to society for treatment of ant stings can be staggering: in the United States $6350 was what it cost (based on my calculations using their data) to treat patients stung by just fire ants during one year on a military base housing 23,000 people (Adams and Lofgren, 1982). Simple arithmetic extrapolations of such estimates yield an annual cost of several million dollars in medical expenses just in the small part of the United States that is inhabited by *S. invicta*.

IX. ANTS: VENOM CHEMISTS AND TOXINOLOGISTS *PAR EXCELLENCE*

Although ant venoms have not generally commanded the same attention as those of other stinging Hymenoptera, they are among the most fascinating and complex of insect venoms. Their hallmark is diversity, diversity of venom form, function, and composition based on the even greater diversity of behaviors, needs, and habitats of ants. The plasticity of venom chemistry and function may be the foundation of the incredible success of ants in most of the world's habitats.

Many ant venoms are composed primarily of alkaloids, formic acid, or terpenes, a radical departure in venom chemistry from the venoms of other arthropods. Other ant venoms appear strikingly similar to bee and wasp venoms in their gross biochemical constitution and pharmacological activities. Nevertheless, we must guard against generalizing too greatly between even these ant venoms and those of bees and wasps. Unique constituents of ant venoms may be important in the field of molecular chemistry, neurophysiology, and toxinology. Even our limited knowledge of ant venoms has provided new tools for the study of neurophysiology (piperidine alkaloids in fire ant venoms), the most toxic insect venom yet known, *Pogonomyrmex*, and a venom with possible potential for understanding or treating rheumatoid arthritis, *Pseudomyrmex*. From the time when an ant venom was the first insect venom chemically analyzed to the present day, the complexities of ant venoms have provided challenges and thrills for scientific investigators. The complexities of ant venom chemistry and action will continue to be a challenge, while the applications of the knowledge will be a human benefit, in years to come.

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